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<p>(21) International Application Number: PCT/EP90/00400 (22) International Filing Date: 13 March 1990 (13.03.90)</p> <p>(30) Priority data: 89105525.3 29 March 1989 (29.03.89) EP (34) Countries for which the regional or international application was filed: GB et al.</p> <p>(71) Applicant (for all designated States except US): GRUPPO LEPETIT S.P.A. [IT/IT]; Via Murat, 23, I-20159 Milano (IT).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only) : MALABARBA, Adriano [IT/IT]; Via Roma, 5/A, I-20082 Binasco (IT). SENECI, Pierfausto [IT/IT]; Via Armando Diaz, 9, I-25121 Brescia (IT). KETTENRING, Jürgen, Kurt [DE/IT]; Via Oronco, 27, I-21100 Varese (IT). CIABATTI, Romeo [IT/IT]; Via Brodolini, 15/A, I-20026 Novate Milanese (IT).</p>		<p>(74) Agent: PIMPINELLI, Natale; Gruppo Lepetit S.p.A., Patent & Trademark Department, Via Roberto Lepetit, 34, I-21040 Gerenzano (IT).</p> <p>(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), SU, US.</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: NEW SUBSTITUTED ALKYLAMIDE DERIVATIVES OF TEICOPLANIN</p> <p>(57) Abstract</p> <p>The present invention is directed to new C⁶³ amide derivatives of teicoplanin wherein the amide moiety is derived from a di- or poly-alkylamine, and to a process for preparing them. The derivatives are prepared by reacting a teicoplanin-like product with an active ester forming reagent such as chloroacetonitrile and then contacting said active ester with the appropriate di- or poly-alkylamine. The amide derivatives of the invention are active against gram positive and gram negative bacteria.</p>			

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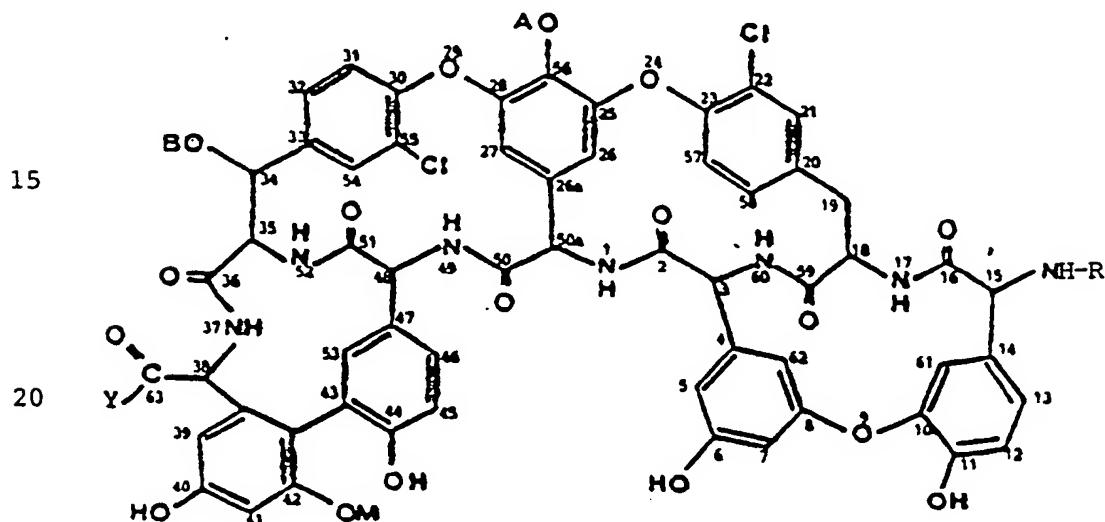
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NEW SUBSTITUTED ALKYLAMIDE DERIVATIVES
OF TEICOPLANIN

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The present invention is directed to substituted
alkylamides of teicoplanin compounds having the
10 following formula I



25

wherein

R represents hydrogen or a protecting group of
30 the amine function;

Y represents a compound of formula

$-NR_1\text{-alk}_1\text{-}\bar{x}\text{-alk}_2\text{-}\bar{p}\text{-}\bar{T}\text{-alk}_3\text{-}\bar{q}\text{-}W$

wherein

R_1 represents hydrogen or $(C_1\text{-}C_4)$ alkyl;

- alk₁, alk₂ and alk₃ each independently represents a linear or branched alkylene of 2 to 10 carbon atoms;
- 5 p represents an integer comprised between 1 and 50;
- 5 q represents an integer comprised between 0 and 12;
- X represents a -NR₂- group or an oxygen atom wherein R₂ represents hydrogen, (C₁-C₄)alkyl, a group alk₄NR₃R₄ wherein alk₄ represents a linear or branched 10 alkylene of 2 to 4 atoms, R₃ is hydrogen or (C₁-C₄)alkyl and R₄ is hydrogen, (C₁-C₄)alkyl or a 5-6 membered cycloalkyl; or R₁ and R₂ taken together represent a (C₂-C₄)alkylene moiety connecting the two nitrogen atoms with the proviso that in such case p is 1;
- 15 T represents a -NR₅- group or an oxygen atom wherein R₅ is hydrogen, (C₁-C₄)alkyl; a group alk₅NR₆R₇ wherein alk₅ represents a linear or branched alkylene of 2 to 4 atoms, R₆ is hydrogen or (C₁-C₄)alkyl and R₇ is hydrogen, (C₁-C₄)alkyl or a 5-6 membered cycloalkyl; or 20 R₂ and R₅ taken together represent a (C₂-C₄)alkylene moiety connecting the two nitrogen atoms with the proviso that in such case both p and q are 1;
- 25 W represents hydroxy, NR₈R₉ wherein R₈ is H or (C₁-C₆)alkyl and R₉ is H, (C₁-C₆)alkyl, a 5-6 membered cycloalkyl, COOR₁₀ wherein R₁₀ represents (C₁-C₆)acyloxy-(C₁-C₄)alkyl, and the group N⁺R₁₁R₁₂R₁₃A⁻ wherein 30 R₁₁, R₁₂ and R₁₃ each independently represents (C₁-C₄)alkyl and A⁻ is an anion derived from a pharmaceutically acceptable acid; with the proviso that when simultaneously X is NR₂, p is 1 and q is zero, then W is different from hydroxy;
- 35 A represents H or -N/(C₉-C₁₂)aliphatic acyl-⁷-beta-D-2-deoxy-2-aminoglucopyranosyl,

B represents hydrogen or
N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl;

M represents hydrogen or alpha-D-mannopyranosyl
5
and the pharmaceutically addition salts thereof;

with the further proviso that B represents hydrogen
only when A and M are simultaneously hydrogen.

10

Teicoplanin is the international non-proprietary
name (INN) of the antibiotic substance formerly named
teichomycin which is obtained by cultivating the strain
15 Actinoplanes teichomyceticus nov. sp. ATCC 31121 in a
culture medium containing assimilable sources of carbon,
nitrogen and inorganic salts (see U.S. Patent
No. 4,239,751).

According to the procedure described in the above
20 cited patent an antibiotic complex containing
Teichomycin A₁, A₂ and A₃ is recovered from the
separated fermentation broth by extraction with a
suitable water insoluble organic solvent and
precipitation from the extracting solvent according to
25 common procedures. Teichomycin A₂, which is the major
factor of the isolated antibiotic complex, is then
separated from the other factors by means of column
chromatography on Sephadex®. British Patent No. 2121401
discloses that antibiotic Teichomycin A₂ actually is a
30 mixture of five closely related co-produced main
components.

According to recent structural studies it is
possible to represent teicoplanin A₂ (formerly
Teichomycin A₂) main components 1, 2, 3, 4 and 5 by the
35 above formula I wherein R is hydrogen, Y is hydroxy, A

represents $-N/(C_{10}-C_{11})$ -aliphatic acyl $\bar{\gamma}$ -beta-D-2-deoxy-2-amino-glucopyranosyl, B represent N-acetyl-beta-D-2-deoxy-2-amino-glucopyranosyl, M represents alpha-D-mannopyranosyl.

5 More particularly, in teicoplanin A₂ component 1, the $/(\bar{C}_{10}-C_{11})$ -aliphatic acyl $\bar{\gamma}$ substituent represents Z-4-decenoyl, in teicoplanin A₂ component 2 represents 8-methyl-nonanoyl, in teicoplanin A₂ component 3 represents decanoyl, in teicoplanin A₂ component 4 represents 10 8-methyldecanoyl, in teicoplanin A₂ component 5 represents 9-methyldecanoyl.

European Patent Application Publication No. 306645 describes production of teicoplanin compounds where the aliphatic acid group of the beta-D-2-deoxy-2-amino-15 -glucopyranosyl moiety is a 6-methyl-octanoyl group (compound A or RS3) or a n-nonanyl group (compound B or RS4).

In the paper entitled: "Isolation by HPLC and 20 structural determination of minor components of teicoplanin" given by Zanol et al., at the 17th International Symposium on chromatography, Wien, September 25-30, 1988, other two teicoplanin compounds (RS1 and RS2) are described.

25 Said compounds are characterized in that the aliphatic acyl moieties of the beta-D-2-deoxy-2-amino-glucopyranosyl moiety are respectively methyl-undecanoyl (RS1) and dodecanoyl (RS2).

All the sugar moieties, when present, are linked to 30 the teicoplanin nucleus through O-glycosidic bonds.

In addition, it has been found that it is possible to transform teicoplanin, a pure factor thereof or a mixture of any of said factors in any proportion, into 35 unitary antibiotic products by means of selective

hydrolysis of one or two sugar moieties. They are named antibiotic L 17054 and antibiotic L 17046 and are described in European Patent No. 119575 and European Patent No. 119574, respectively.

5 Preferred hydrolysis conditions for the production of antibiotic L 17054 are: 0.5 N hydrochloric acid at a temperature between 70°C and 90°C and for a time which is generally between 15 and 90 min.

Antibiotic L 17054 is represented by the above
10 formula I wherein Y is hydroxy, R and A represent hydrogen, B represents N-acetyl-beta-D-2-deoxy-2-amino-glucopyranosyl, M represents alpha-D-mannopyranosyl wherein the sugar moieties are linked to the peptidic nucleus through an O-glycosidic bond.

15

Preferred hydrolysis conditions for the preparation of antibiotic L 17046 are: 1-3 N hydrochloric acid, at a temperature between 50° and 90°C and for a time which is generally between 30 and 60 min.

20

Antibiotic L 17046 is represented by the above formula I wherein Y is hydroxy, R, A and M represent hydrogen atoms, and B is N-acetyl-beta-D-2-deoxy-2-amino-glucopyranosyl wherein the sugar moiety is linked to the peptidic nucleus through an O-glycosidic bond.

25

European Patent Application publication No. 301247 describes de-mannosyl teicoplanin derivatives, i.e. compounds of the formula I above wherein A and B are different from hydrogen, M is hydrogen and Y is hydroxy.

30

The complete selective cleavage of all the sugar moieties of the teicoplanin compounds gives an aglycone molecule which is called antibiotic L 17392, or deglucoteicoplanin, and is represented by the above
35 formula I wherein Y is hydroxy, and R, A, B, and M each

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individually represents a hydrogen atom. This selective hydrolysis process is described in European patent application publication No. 146053.

5 A substance having the same structural formula is disclosed in European Patent Application Publication No. 0090578 and is named antibiotic A 41030 factor B.
10 This substance is obtained by means of a micro-
biological process which involves the fermentation of
Streptomyces virginiae NRRL 15156 in a suitable medium,
the isolation, purification and separation into its
components of antibiotic A 41030, an antibiotic complex
of at least seven factors, antibiotic A 41030 factor B,
included.

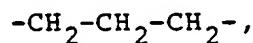
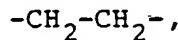
15 All the above named compounds, i.e. teicoplanin,
teicoplanin A₂ complex, teicoplanin A₂ component 1,
teicoplanin A₂ component 2, teicoplanin A₂ component 3,
20 teicoplanin A₂ component 4, teicoplanin A₂ component 5,
"compound A or RS3", "compound B or RS4", RS1, RS2,
antibiotic L 17054, antibiotic L 17046, antibiotic
L 17392, the de-mannosyl teicoplanin derivatives of
25 European Patent Application publication No. 301247, and
any mixture thereof in any proportion, are suitable
starting materials for the preparation of the
substituted alkylamide derivatives of the invention.
30 In the present specification "teicoplanin compound"
or "teicoplanin starting material" is used to indicate
any one of the above starting materials, i.e.
teicoplanin as obtained according to U.S. patent
35 4,239,751, any further purification thereof, teicoplanin
A₂ complex, a compound of the above formula I wherein R
is hydrogen or an N-protecting group, Y is hydroxy, A
represents hydrogen or -N/[C₉-C₁₂]aliphatic

acyl⁷-beta-D-2-deoxy-2-aminoglucopyranosyl, B represents hydrogen or N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl, M represents hydrogen or alpha-D-mannopyranosyl, with the proviso that B may represent hydrogen only when 5 A and M are simultaneously hydrogen, a salt thereof, or a mixture thereof in any proportion.

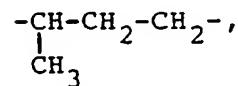
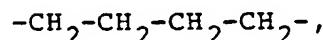
As used herein the term "alkyl", either alone or in combination with other substituents, includes both 10 straight or branched hydrocarbon groups; more particularly, "(C₁-C₆)alkyl" represents a straight or branched aliphatic hydrocarbon chain of 1 to 6 carbon atoms such as methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 1,1-dimethylethyl, pentyl, 15 1-methylbutyl, 2-methylbutyl, 1-hexyl, 2-hexyl, 3-hexyl, 3,3-dimethyl-1-butyl, 4-methyl-1-pentyl and 3-methyl-1-pentyl; likewise, "(C₁-C₄)alkyl" represents a straight or branched hydrocarbon chain of 1 to 4 carbon atoms such as those alkyl of 1 to 4 carbons exemplified 20 above.

As used herein the terms "alk₁", "alk₂", "alk₃", represent an independent linear or branched alkylene chain of 2 to 10 carbon atoms such as for example:

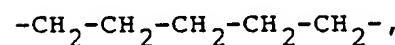
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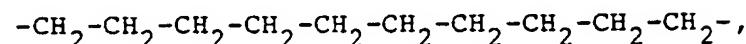
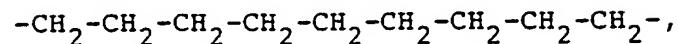
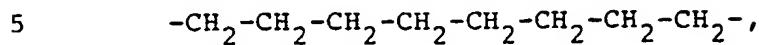
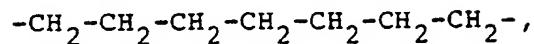
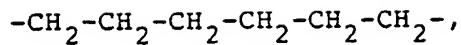


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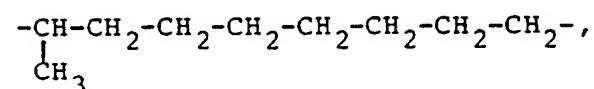


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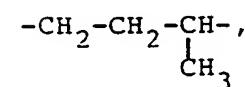
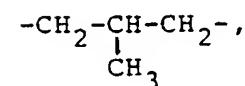




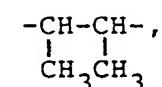
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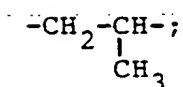
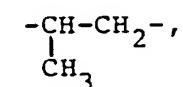
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30 likewise, "alk₄" and "alk₅" represent an independent linear or branched alkylene chain of 2 to 4 carbon atoms as those alkylene of 2 to 4 carbon atoms exemplified above.

35 Preferred compounds are those of formula I wherein:

R represents hydrogen or a protecting group of the amine function;

Y represents a compound of formula

-NR₁-alk₁-/⁻X-alk₂-/⁻p-/⁻T-alk₃-/⁻q-W

wherein

R₁ represents hydrogen or (C₁-C₄)alkyl;

alk₁, alk₂ and alk₃ each independently represents a linear or branched alkylene of 2 to 4 carbon atoms;

p represents an integer comprised between 1 and 10 12;

q represents an integer comprised between 0 and 12;

X represents a -NR₂- group or an oxygen atom wherein R₂ represents hydrogen, (C₁-C₄)alkyl, a group alk₄NR₃R₄ wherein alk₄ represents a linear or branched alkylene of 2 to 4 atoms, R₃ is hydrogen or (C₁-C₄)alkyl and R₄ is hydrogen, (C₁-C₄)alkyl or a 5-6 membered cycloalkyl; or R₁ and R₂ taken together represent a (C₂-C₄)alkylene moiety connecting the two nitrogen atoms 20 with the proviso that in such case p is 1;

T represents a -NR₅- group or an oxygen atom wherein R₅ is hydrogen, (C₁-C₄)alkyl a group alk₅NR₆R₇ wherein alk₅ represents a linear or branched alkylene of 2 to 4 atoms, R₆ is hydrogen or (C₁-C₄)alkyl and R₇ is 25 hydrogen, (C₁-C₄)alkyl or a 5-6 membered cycloalkyl; or R₂ and R₅ taken together represent a (C₂-C₄)alkylene moiety connecting the two nitrogen atoms with the proviso that in such case both p and q are 1;

W represents hydroxy, NR₈R₉ wherein R₈ is H or 30 (C₁-C₆)alkyl and R₉ is H, (C₁-C₆)alkyl, a 5-6 membered cycloalkyl, COOR₁₀ wherein R₁₀ represents (C₁-C₆)acyloxy-(C₁-C₄)alkyl, and the group N⁺R₁₁R₁₂R₁₃AN wherein

R₁₁, R₁₂ and R₁₃ each independently represents 35 (C₁-C₄)alkyl and AN is an anion derived from a

pharmaceutically acceptable acid; with the proviso that when simultaneously X is NR₂, p is 1 and q is zero, then W is different from hydroxy;

5 A represents H or -N/[C₉-C₁₂)aliphatic acyl]-beta-D-2-deoxy-2-aminoglucopyranosyl,

B represents hydrogen or N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl;

10 M represents hydrogen or alpha-D-mannopyranosyl, and the pharmaceutically addition salts thereof;

15 with the further proviso that B represents hydrogen only when A and M are simultaneously hydrogen.

Preferably, when X and/or T represent -NR₂- and/or -NR₅- then alk₄ and alk₅ represent a C₂-C₃ linear chain.

20 As described above p is an integer comprised between 1 and 50 and q is an integer comprised between 0 and 12. Preferably, when X and/or T represent -NR₂- and/or -NR₅- atoms p and q are comprised between 1 and 12, while when both X and T represent oxygen atoms, p and q are such that p+q is comprised between 2 and 50.

25 The term "C₅-C₆ cycloalkyl" as used in the specification and claims refers to the cyclopentyl and cyclohexyl group optionally substituted with 1 to 3 lower alkyl such as methyl and ethyl.

30 Preferred compounds are those of formula I wherein X represents a -NR₂- group wherein R₂ is hydrogen, a (C₁-C₄)alkyl or a alk₄NR₃R₄.

Another group of preferred compounds are those of formula I wherein p is 1 and X is $-NR_2-$ wherein R_2 taken together with R_1 represent a (C_2-C_3) alkylene moiety connecting the nitrogen atoms.

5 In such case are particularly preferred those compounds wherein alk₁ represents the group $-CH_2-CH_2-$.

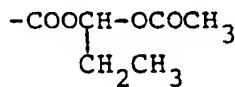
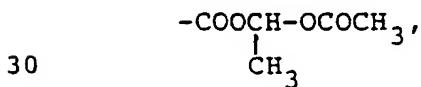
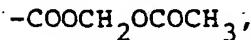
A further preferred group of compounds are those of formula I wherein p is 1, q is 1 and X and T are $-NR_2-$ and $-NR_5-$ respectively, wherein R_2 and R_5 taken together 10 represent a (C_2-C_3) alkylene moiety connecting the nitrogen atoms.

In such case are particularly preferred those compounds wherein alk₂ represents the group $-CH_2-CH_2-$.

Other preferred compounds are represented by 15 formula I wherein X and T are oxygen atoms, p+q is comprised between 2 and 50 and W is hydroxy or NR_8R_9 , wherein R_8 is hydrogen or (C_1-C_4) alkyl and R_9 is hydrogen, (C_1-C_4) alkyl, cyclopentyl or cyclohexyl.

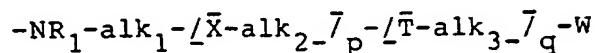
Further preferred compounds are those wherein W 20 represents NR_8R_9 wherein R_8 is as defined and R_9 is $COOR_{10}$ being R_{10} a (C_1-C_6) acyloxy- (C_1-C_4) alkyl group.

In the term " (C_1-C_6) acyloxy- (C_1-C_4) alkyl" the group (C_1-C_4) alkyl is a methylene moiety optionally substituted with a (C_1-C_3) linear or branched alkyl chain 25 such as for example:



35 and the like.

According to the general definitions given above representative examples of the group:

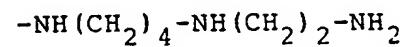
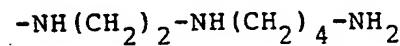
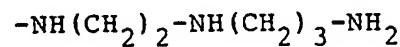


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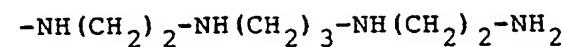
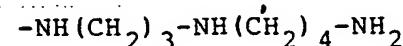
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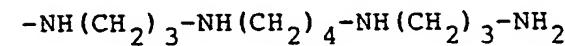
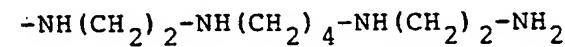
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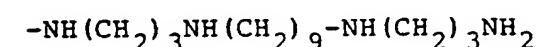
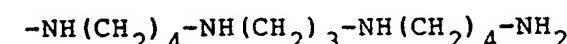
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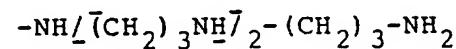
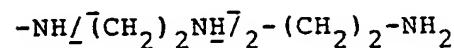
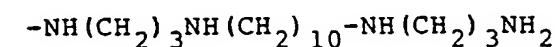
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30



35

$$-\text{NH}-\overline{(\text{CH}_2)}_4\text{NH}\overline{(\text{CH}_2)}_5-$$

$$-\text{NH}_2 - (\text{CH}_2)_5 \text{NH} - (\text{CH}_2)_5 - \text{NH}_2$$

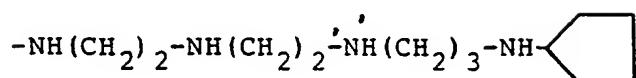
5 $\text{--NH}(\text{CH}_2)_3\overset{\text{N}}{\underset{(\text{CH}_2)_3\text{NH}_2}{\underset{|}{\text{N}}}}(\text{CH}_2)_3\text{N}(\text{CH}_3)_2$

$$-\text{NH}(\text{CH}_2)_3\underset{(\text{CH}_2)_3\text{NH}_2}{\underset{|}{\text{N}}}(\text{CH}_2)_3\text{N}(\text{C}_2\text{H}_5)_2$$

$$-\text{NH}(\text{CH}_2)_2-\text{NH}(\text{CH}_2)_2-\text{NHCH}_3$$

$$-\text{NH}(\text{CH}_2)_2-\text{NH}(\text{CH}_2)_2-\text{NHC}_2\text{H}_5$$

15 $-\text{NH}(\text{CH}_2)_2-\text{NH}(\text{CH}_2)_4-\text{NH}(\text{nC}_4\text{H}_9)$

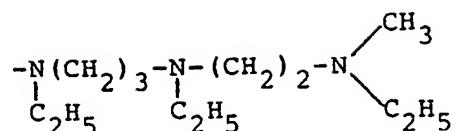


$$-\underset{\text{CH}_3}{\text{N}}(\text{CH}_2)_2-\text{NH}- (\text{CH}_2)_4 - \underset{\text{CH}_3}{\text{N}}-\text{C}_6\text{H}_{11}$$

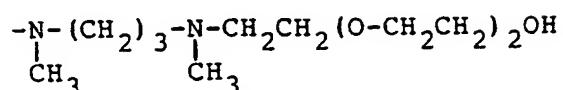
25 $\text{--NH}(\text{CH}_2)_3\text{N}^+(\text{CH}_2)_3\text{NH}_2^+$

30  The structure shows a central nitrogen atom bonded to three methyl groups and one ethyl group. The ethyl group is further substituted with a benzyl group.

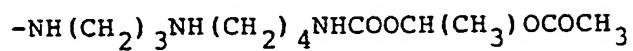
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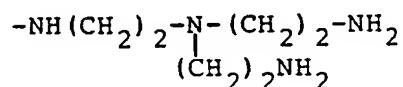
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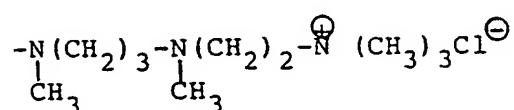
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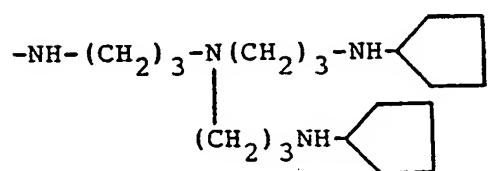
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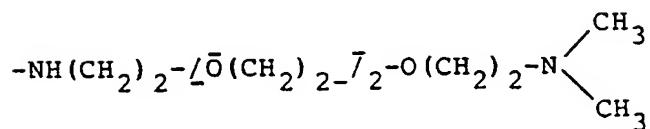
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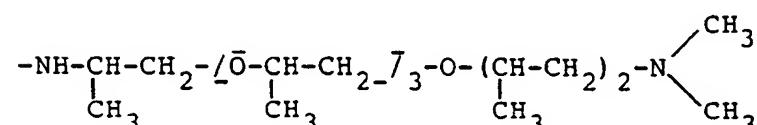
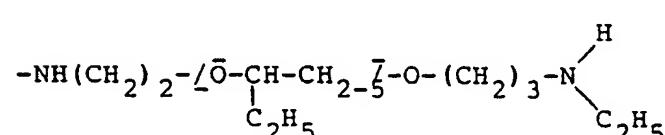
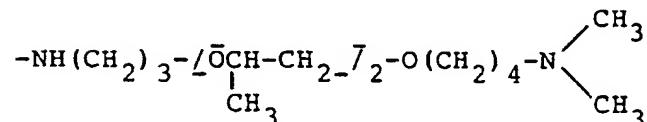
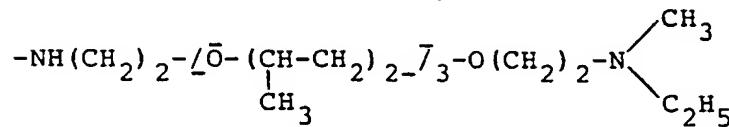
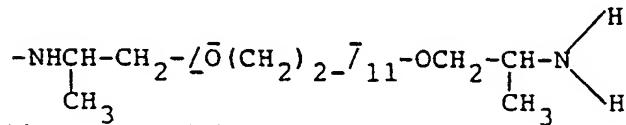
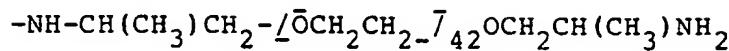
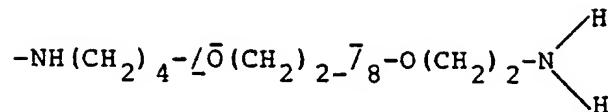
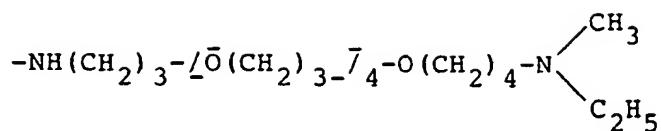


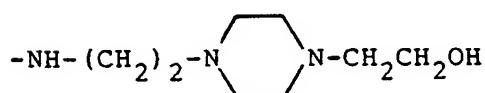
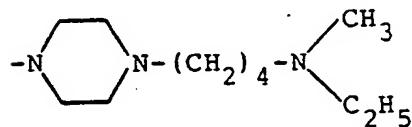
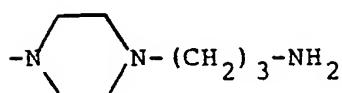
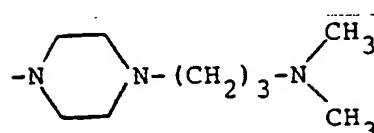
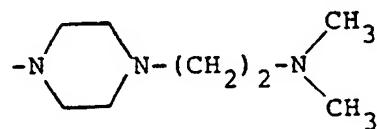
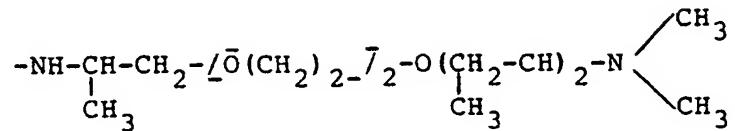
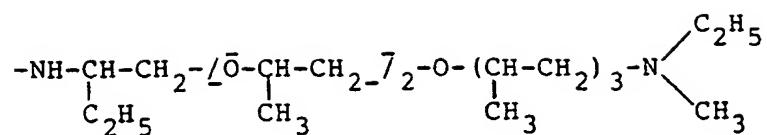
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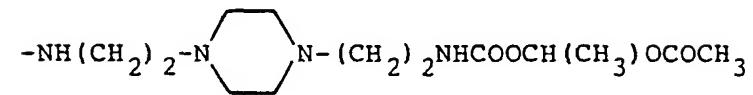
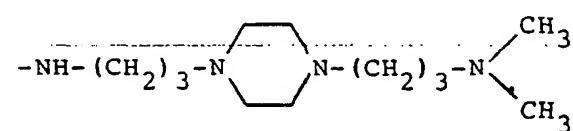
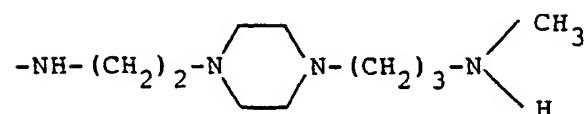
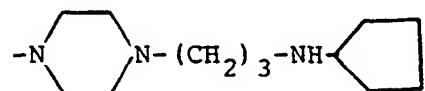


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The compounds of this invention show antimicrobial activity and are useful as semi-synthetic antibacterial agents against gram positive bacteria but are also particularly active against gram negative bacteria and more particularly against Escherichia coli and Pseudomonas aeruginosa.

Various C⁶³ amide derivatives of teicoplanin complex, single components and the aglycon and pseudoaglycons thereof are described in European patent

application publication No. 218099 and International Patent application publication No. WO 88/06600.

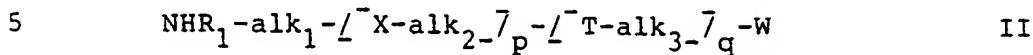
The compounds of the present invention are prepared
5 by amidation of the corresponding derivatives of the formula I wherein Y is OH, (i.e. the corresponding carboxy acids).

The substances used as starting materials for the manufacture of the compounds of this invention described above, can be either individual products or mixtures of one or more products.

Since said starting materials for the preparation of the compounds of the present invention can be used in both said forms, the resulting end products may, in turn, be individual compounds or mixtures of two or more compounds of the above formula I. These mixtures of compounds are also part of the invention and may be used as such for their biological applications and uses or 20 may be eventually separated in their individual components by known procedures described in the art. Examples of separation procedures suitable for the purpose of obtaining individual components from end products mixtures of teicoplanin amide derivatives are 25 those described in the following documents: European Patent Applications Publication No. 218099 and International Patent Application Publication No. WO 88/06600.

30 The amidation procedures described in the two above mentioned European Patent Application and International Patent Application can be used also for the preparation of the compounds of this invention. Said procedures involve condensing the carboxy acid starting materials

mentioned above with an excess of the appropriate amine of the formula II:



wherein R_1 , alk_1 , alk_2 , alk_3 , X , T , p , q and W have the same meanings as above, in an inert organic solvent in the presence of a condensing agent.

10 Inert organic solvents useful for the amidation reaction are those organic aprotic solvents which do not unfavorably interfere with the reaction course and are capable of at least partially solubilizing the teicoplanin starting material.

15 Examples of said inert organic solvents are organic amides, alkyl ethers, ethers of glycols and polyols, phosphoramides and sulfoxides. Preferred examples of inert organic solvents are: dimethylformamide, dimethoxyethane, hexamethylphosphoramide, dimethylsulfoxide and mixtures thereof.

20 The condensing agent in the process of the invention is one suitable for forming amide bonds in organic compounds and in particular in peptide synthesis.

25 Representative examples of condensing agents are (C_1-C_4) alkyl, phenyl or heterocyclic phosphorazidates such as, diphenyl phosphorazidate, diethyl phosphorazidate, di(4-nitrophenyl) phosphorazidate, dimorpholyl-phosphorazidate and diphenylphosphorochloridate. The preferred condensing agent is diphenyl phosphorazidate, i.e. phosphoric acid diphenyl ester azide (DPPA). In the amidation process of the invention described here, the amine reactant is normally used in a molar excess.

In general, when the amine reactant is a fairly unexpensive or easily obtainable reactant, a 2- to 6-fold molar excess is used while a 3 to 4-fold molar excess is preferred.

5 For the amidation to proceed, it is necessary that the amine be capable of forming a salt with the carboxy function of the teicoplanin starting material. In case the amine is not strong enough to form such a salt in the selected reaction medium, it is necessary to add a
10 salt-forming base to the reaction mixture at least in an equimolecular amount with the teicoplanin starting material.

15 Use of a low molar excess of the amine reactant with addition of a salt-forming base is a suitable method when the amine reactant is a rather expensive or hardly obtainable' product.

20 Examples of said salt-forming bases are tertiary organic aliphatic or heterocyclic amines such as trimethylamine, triethylamine, N-methyl pyrrolidine or picoline, and the like.

The condensing agent is generally employed in a slight molar excess such as from 1.2 to 1.7 times and preferably 1.5 times the teicoplanin starting compound.

25 In addition, the amine reactant may also conveniently be introduced in the reaction medium as a corresponding acid addition salt, e.g. the hydrochloride. In this case, at least a double molar proportion and preferably a 2 to 4 fold molar excess of a strong base capable of freeing the amine from its salts, is used. Also in this
30 case, the suitable base is a tertiary organic aliphatic or heterocyclic amine like those exemplified above. In fact, at least in some instances, the use of a salt of the amine which is then freed in situ with the above mentioned bases, is highly preferred, especially when

the salt is more stable than the corresponding free amine.

The reaction temperature will vary considerably
5 depending on the specific starting materials and reaction conditions. In general, it is preferred to conduct the reaction at temperatures between 0-20°C.

Also the reaction time will vary considerably depending on the other reaction parameters. In general,
10 the condensation reaction is completed in about 24-48 h.

In any case, the reaction course is monitored by TLC or preferably by HPLC according to methods known in the art.

On the basis of the results of these assays a man skilled in the art will be able to evaluate the reaction course and decide when to stop the reaction and start working up the reaction mass according to known per se techniques which include, for instance, extraction with solvents, precipitation by addition of non-solvents, etc., in conjunction with further common separation operations and purifications, e.g. by column chromatography.

If the amine reactant contains other functions
25 which are not inert under the selected reaction conditions, said functions are suitably protected by means of per se known protecting groups.

According to a further preferred embodiment of this invention, the compounds of formula I wherein Y is a group as defined above can be prepared by reacting an "activated ester" of the carboxylic acid of the same formula I, wherein Y is OH and the N¹⁵ -amino function is preferably protected, with an appropriate amine of formula II.

The N¹⁵-amino function can be protected by methods known per se in the art such as those described in reference books like T.W. Greene, "Protective Groups in Organic Synthesis", John Wiley and Sons, New York, 1981, and M. Mc. Omie, "Protecting Groups in Organic Chemistry" Plenum Press, New York, 1973.

The protecting groups must be stable at the conditions of the reaction process, must not unfavorably interfere with the amidation reaction, and must be easily cleavable and removable from the reaction medium at the end of the reaction without altering the newly formed amide bond and the overall structure of the compounds, e.g. sugar components.

Representative examples of N-protecting groups which may be advantageously used in the process of the invention for protecting the N¹⁵ primary amino function of the teicoplanin starting material and, when appropriate, the amino function of the amine II reactant, are carbamate forming reagents characterized by the following oxy carbonyl groups: 1,1-dimethylpropynylloxycarbonyl, t-butyloxycarbonyl, vinyloxycarbonyl, aryloxycarbonyl, cinnamyloxycarbonyl, benzylloxycarbonyl, p-nitrobenzylloxycarbonyl, 3,4-dimethoxy-6-nitrobenzylloxycarbonyl, 2,4-dichlorobenzyl oxy carbonyl, 5-benzisoxazolylmethyloxycarbonyl, 9-anthranyl methyloxycarbonyl, diphenylmethyloxycarbonyl, isonicotinyloxycarbonyl, diphenylmethyloxycarbonyl, isonicotinyloxycarbonyl, S-benzyloxycarbonyl, and the like.

Other suitable N-protecting agents are aldehydes or ketones, or derivatives thereof which are capable of forming Schiff bases with the amino group to be protected.

Preferred examples of such Schiff base forming agents are benzaldehydes and particularly preferred is 2-hydroxybenzaldehyde (salicylaldehyde).

A convenient mean of protection is, in some instances, the formation of a benzylidene derivative which may be prepared by reacting the amine with benzaldehyde in a lower alkanol, such as ethanol, 5 preferably at room temperature. After the reaction with the selected teicoplanin starting material has been completed, the benzylidene protecting group may be removed as known in the art, e.g. by catalytic hydrogenation, using, for instance, Palladium on carbon 10 as the catalyst.

In this case, however, attention should be paid to the presence of groups which may be modified by catalytic hydrogenation. A typical consequence of the catalytic hydrogenation of an amino-protected derivative 15 of formula I wherein A represents a group as above defined whose acyl portion is (Z)-4-decenoyl (or a, mixture containing it) is that, at least partially, the decenoyl compound is transformed into the corresponding decanoyl compound.

20 As it is appreciated by the skilled technician, the ultimate choice of the specific protecting group depends on the characteristics of the particular amide derivative which is desired. In fact, this amide function of the final compound should be stable at the 25 condition of removal of the protecting group(s).

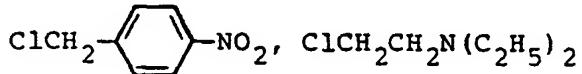
Since the conditions of removal of the different protecting groups are known, the skilled technician is capable of selecting the proper protecting group.

The formation of "activated esters" is described in 30 general terms in Fieser and Fieser, Reagent for organic synthesis, John Wiley and Sons Inc., pages 129-130 (1967).

Examples of said activated ester forming reagents that can be conveniently used in the process of the

invention are those described by R. Schwyzer et al. in Helv. Chim. Acta, 1955, 38, 69-70 and encompass:
 ClCH_2CN , $\text{BrCH}_2\text{COOC}_2\text{H}_5$, $\text{BrCH}(\text{COOC}_2\text{H}_5)_2$, $\text{ClCH}_2\text{COCH}_3$,

5



A preferred reagent of this type is
10 chloroacetonitrile. In this case, chloroacetonitrile itself or dimethylformamide (DMF) can be used as preferred solvents.

Generally, inert organic solvents useful for the formation of "activated esters" are those organic
15 aprotic solvents which do not unfavorably interfere with the reaction course and are capable of, at least partially, solubilizing the carboxyacid starting material.

Examples of said inert organic solvents are organic
20 amides, alkyl ethers, ethers of glycols and polyols, phosphoramides, sulfoxides and aromatic compounds. Preferred examples of inert organic solvents are: dimethylformamide, dimethoxyethane, hexamethylphosphoramide, dimethylsulfoxide, benzene,
25 toluene and mixtures thereof.

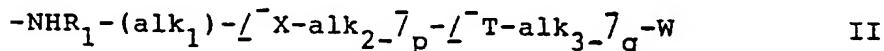
More preferably, the solvent is selected from acetonitrile, dimethylsulfoxide, dimethylformamide. The formation of the activated ester is generally conducted in the presence of a base which does not interfere with
30 the reaction course such as a tri-alkylamine like triethylamine, sodium or potassium carbonate or bicarbonate. Generally, the base is employed in a 2 to 6 molar proportion to the teicoplanin carboxy acid starting material and, preferably, it is used in an

about three-fold molar excess. A preferred base is triethylamine.

The "activated ester" forming reagent is used in a large excess over the teicoplanin carboxy acid starting material. It is in general used in a 5 to 35 molar proportion and preferably, it is used in an about 20 to 30 times molar excess. The reaction temperature is between 10°C and 60°C and preferably between 15°C and 30°C. As usual, the reaction time depends on the other specific reaction parameters and may be generally between 3 and 48 hours.

In this case, the reaction course may be followed by HPLC or TLC to determine when the reaction may be considered as completed and the procedures to recover the desired intermediate can be started. The "activated ester" intermediate can be directly used in the same reaction medium where it is prepared, however, in general, it is isolated by precipitation with non-solvents or by extraction with solvents and it is used as such, without further purification, in the next reaction step. If desired, however, it may be purified by column chromatography such as flash column chromatography or reverse-phase column chromatography.

The obtained "activated ester" intermediate is then reacted with a molar excess of the amine derivative of formula II



30

in the presence of an organic polar solvent at a temperature between 5°C and 60°C, preferably between 10°C and 30°C.

35 The organic polar solvent can be in this case a polar protic solvent or an aprotic one.

Preferred examples of organic polar protic solvents are lower (C_2 - C_4) alkanols such as, ethanol, n-propanol, iso-propanol, n-butanol and the like, or mixtures thereof, preferably used in the dry form.

5 Preferred examples of organic polar aprotic solvent are N,N-dimethylformamide (DMF), hexamethylphosphoramide (HMPA), or mixtures thereof, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)pyrimidone (DMPU), dimethylsulfoxide (DMSO) or dimethoxyethane (DME).

10 The reaction of the "activated ester" with the selected amine can be carried out at a temperature between 5°C and 60°C but the preferred temperature is generally comprised between 10°C and 30°C, most preferably between 20°C and 25°C, while a preferred 15 molar proportion between the "activated ester" intermediate and the amine II as above defined is from 1:5 to 1:30, and more preferably from 1:10 to 1:20. The reaction course may be monitored as usual by TLC or HPLC.

20 The amide derivative obtained from the amidation reaction is recovered from the reaction solution according to common procedures, for instance, by evaporation of the solvent or by addition of a non-solvent. The removal of the amino-protecting group 25 is usually carried out on the crude product isolated from the amidation reaction.

Examples of procedures for the removal of said protecting groups from teicoplanin derivatives are described for instance in International Application 30 Publication No. WO 88/06600.

If catalytic hydrogenation procedures are used, the reaction is usually carried out in the presence of a diluted aqueous strong acid, preferably a mineral acid, in an organic solvent miscible with said diluted aqueous 35 strong acid. The filtrate from the reaction is then

worked for the recovery of either the mineral acid addition salt of the amide of formula I or the corresponding free base. Analogous procedures are followed when the amino-protecting group is a group
5 which can be removed by treating with diluted mineral acids (e.g. Schiff base or a C₁-C₄ alkoxy carbonyl group) under conditions which do not cause the splitting of the sugar moieties (e.g. low temperatures, short reaction time).

10

A further procedure for the preparation of a compound of formula I of this invention consists in reacting an N¹⁵ protected derivative of a N⁶³ amide of formula I wherein Y is -NR₁alk₁XH or
15 NR₁-alk₁-/ \bar{X} -alk₂-⁷_p-TH with a reactant of the formula r-/ \bar{alk}_2 -⁷_p-/ \bar{T} -alk₃-⁷_q-W or r-/ \bar{alk}_3 -⁷_q-W, respectively, wherein the symbol R₁, alk₁, alk₂, alk₃, X and T are the same as above, \bar{x} represents halo, methanesulfonyl or tosyl, in the presence of an acid acceptor in an inert
20 solvent. In such cases, p is preferably 1 or 2, q is different from zero and preferably is 1 or 2, X and T preferably represent NH or oxygen, most preferably oxygen. The N¹⁵ protected derivative of the N⁶³ amide referred above are prepared according to the general
25 method for the preparation of the compounds of formula I of this invention.

When a compound of formula I wherein W represents -NR₈R₉- wherein R₈ is defined as above, R₉ is COOR₁₀ and
30 R₁₀ is a (C₁-C₆)acyloxy-(C₁-C₄)alkyl is desired, it is necessary to react a N¹⁵ protected derivative of the N⁶³ amide wherein W is -NHR₈- being R₈ defined as above with an alpha-acyloxy-alkyl para-nitrophenyl carbonate in the presence of an anhydrous alkaline carbonate such
35 as sodium carbonate.

The alpha-acyloxy-alkyl para-nitrophenyl carbonate can be prepared as described in J. Med. Chem., 31, pages 318-322 (1988).

5 Some amides of the present invention, such as those of teicoplanin A₂ complex, a single component thereof or any mixture of two or more of said components can be used as the starting material for preparing unitary antibiotic products by means of selective hydrolysis of
10 one or two sugar moieties by following the procedure described in the already cited European Patent No. 119575 and European Patent No. 119574.

An alternative method for the manufacture of
15 compounds of formula I wherein A is hydrogen, B is N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl and M is alpha-D-mannopyranosyl consists in hydrolyzing the corresponding amide compounds of formula I wherein A is -N/(C₉-C₁₂) aliphatic acyl-beta-D-2-deoxy-2-aminoglu-
20 copyranosyl, B is N-acetyl-beta-D-2-deoxy-2-aminoglu- copyranosyl and M is alpha-D-mannopyranosyl (i.e. the carboxyamide derivatives of teicoplanin A₂ complex or a single component thereof) according to the process described in the European Patent Application Publication
25 No. 146822.

The process consists in contacting the above material with concentrated aqueous organic acid at about room temperature, preferably with aqueous trifluoroacetic acid at a concentration between 75% and
30 95% at a temperature comprised between 10°C and 50°C.

An alternative process for preparing the compounds of formula I wherein both A and M are hydrogen and B is N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl consists
35 in submitting the compounds of formula I wherein A is

N/₁(C₉-C₁₂)aliphatic acyl⁷-beta-D-2-deoxy-2-aminogluco-pyranosyl, B is N-acetyl-beta-D-2-deoxy-2-aminogluco-pyranosyl and M is alpha-D-mannopyranosyl to an hydrolysis process according to European Patent

5 Application Publication No. 175100.

The process consists in contacting the above starting material with a strong acid in the presence of a polar aprotic organic solvent selected from ethers, ketones and mixture thereof which are liquid at room
10 temperature.

In this latter case as the starting materials can be used also the amide compounds of formula I wherein A is hydrogen, B is N-acetyl-beta-D-2-deoxy-2-aminogluco-pyranosyl and M is alpha-D-mannopyranosyl which are
15 obtained according to the hydrolysis process with concentrated aqueous trifluoroacetic acid as described above.

For the isolation of the acid addition salt, the
20 reaction solution resulting from the splitting of the amino-protecting group is generally brought to a pH value between 4 and 7 by addition of an aqueous base, e.g. aqueous sodium hydroxide, and, after evaporation of the solvent under reduced pressure, the resulting solid
25 is separated in the form of an addition salt with the strong acid which has been added during the de-protection step. Such product may be further purified by common techniques e.g. column chromatography, precipitation from solutions by addition of
30 non-solvents, preparative HPLC and similar. The acid addition salt may be converted to the corresponding free base of formula I by suspending or dissolving the acid addition salt in an aqueous solvent which is then brought to an appropriate pH value whereby the free-base
35 form is restored. The product is then recovered, for

instance, by extraction with an organic solvent or is transformed into another acid addition salt by adding the selected acid and working up as above.

Sometimes, after the above operation, it may be
5 necessary to submit the recovered product to a common
desalting procedure.

For example, column chromatography on controlled
pore polydextrane resins (such as Sephadex LH 20) or
silanized silica gel may be conveniently used. After
10 eluting the undesired salts with an aqueous solution,
. the desired product is eluted by means of linear
gradient or step-gradient of a mixture of water and a
polar or apolar organic solvent, such as
acetonitrile/water from 5% to about 100% acetonitrile
15 and then recovered by evaporation of the solvent or by
lyophilization.

A compound of formula I in the free-base form can
be transformed into the corresponding acid addition salt
by suspending or dissolving the free base form in an
20 aqueous solvent and adding a slight molar excess of the
selected acid. The resulting solution or suspension is
then lyophilized to recover the desired acid addition
salt. Instead of lyophilizing, in some instances, it is
possible to recover the final salt through precipitation
25 by addition of a non-solvent mixable with water.

In case the final salt is insoluble in an organic
solvent where the free base form is soluble it may be
recovered by filtration from the organic solution of the
non-salt form after addition of the stoichiometric
30 amount or a slight molar excess of the selected acid.

Representative and suitable acid addition salts of
the compounds of formula I include those salts formed by
standard reaction with both organic and inorganic acids
such as, for example, hydrochloric, hydrobromic,
35 sulfuric, phosphoric, acetic, trifluoroacetic,

trichloroacetic, succinic, citric, ascorbic, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, 5 benzenesulfonic, sorbic, picric, benzoic, cinnamic and the like acids.

Preferred addition salts of the compounds of this invention are the pharmaceutically acceptable acid addition salts.

10 With the term "pharmaceutically acceptable acid addition salts" are intended those salts with acids which from biological, manufacturing and formulation standpoint are compatible with the pharmaceutical practice.

15 Example of acids suitable for the "pharmaceutically acid addition salts," includes those listed above.

20 The compounds of the invention in the form of both the free bases and their acid addition salts are useful as antibacterial agents, both against gram-positive and gram-negative bacteria.

However, the compounds of the invention show a striking good activity against gram-negative bacteria, more particularly against Pseudomonas aeruginosa.

25 In fact, at present, they are the most active derivatives among teicoplanin antibiotics against the microorganisms of this genus. Said activity is particularly relevant for those compounds of the invention having a deglucoteicoplanin core but is 30 remarkable also for the compounds of the invention which have a teicoplanin nucleus.

35 The antibacterial activity of the compounds of the invention can be demonstrated in vitro by means of

standard two-fold dilution tests in microtiter, using Difco Todd-Hewitt broth (Strep. pyogenes and Strep. pneumoniae) or Oxoid Iso-Sensitest broth (Staphylococci, Strep. faecalis, and gram-negative organisms). Broth cultures are diluted enough so that the final inoculum is about 10^4 colony forming units/ml (CFU/ml). Minimal inhibitory concentration (MIC) is considered as the lowest concentration which shows no visible growth after 18-24 h incubation at 37°C.

10

The results of the antibacterial testing of representative compounds of the present invention are summarized in Table I.

TABLE I
In vitro activity (MIC microgram/ml)

Test Organisms	Compounds No.				
	6	9	11	21	22
<u>Staphylococcus aureus</u> TOUR	0.12	0.12	0.12	0.06	0.06
<u>Staphylococcus epidermidis</u> ATCC 12228	0.06	0.06	0.06	0.06	0.03
<u>Staphylococcus haemolyticus</u> L 602	0.12	0.12	0.12	0.12	0.12
<u>Streptococcus pyogenes</u> C 203	0.06	0.06	0.06	0.12	0.06
<u>Streptococcus pneumoniae</u> UC 41	0.12	0.12	0.12	0.12	0.12
<u>Streptococcus faecalis</u> ATCC 7080	0.12	0.12	0.12	0.12	0.12
<u>Escherichia coli</u> SKF 12140	>128	>128	64	8	1
<u>Proteus vulgaris</u> X19H ATCC 881	>128	>128	128	64	32
<u>Pseudomonas aeruginosa</u> ATCC 10145	64	64	32	64	8

TABLE I (continued)
In vitro activity (MIC microgram/ml)

Test Organisms	Compounds No.					
	23	24	25	26	27	
<u>Staphylococcus aureus</u> TOUR	0.06	0.06	0.12	0.06	0.06	0.06
<u>Staphylococcus epidermidis</u> ATCC 12228	0.06	0.06	0.06	0.016	0.06	0.06
<u>Staphylococcus haemolyticus</u> L 602	0.06	0.06	0.12	0.06	0.12	L
<u>Streptococcus pyogenes</u> C 203	0.06	0.06	0.06	0.06	0.06	0.06
<u>Streptococcus pneumoniae</u> UC 41	0.12	0.12	0.12	0.12	0.12	0.12
<u>Streptococcus faecalis</u> ATCC 7080	0.12	0.12	0.12	0.25	0.12	
<u>Escherichia coli</u> SKF 12140	2	4	2	0.5	2	
<u>Proteus vulgaris</u> X19H ATCC 881	32	128	64	64	64	
<u>Pseudomonas aeruginosa</u> ATCC 10145	8	64	16	2	8	

TABLE I (continued)
In vitro activity (MIC microgram/ml)

Test Organisms	Compounds No.				
	29	30	34	39	44
<u>Staphylococcus aureus</u> TOUR	0.06	0.06	0.06	0.25	0.12
<u>Staphylococcus epidermidis</u> ATCC 12228	0.016	0.06	0.06	0.06	0.06
<u>Staphylococcus haemolyticus</u> L 602	0.06	0.12	0.12	0.12	0.06
<u>Streptococcus pyogenes</u> C 203	0.06	0.06	0.06	0.12	0.06
<u>Streptococcus pneumoniae</u> UC 41	0.12	0.12	0.12	0.5	0.25
<u>Streptococcus faecalis</u> ATCC 7080	0.12	0.12	0.12	0.5	0.25
<u>Escherichia coli</u> SKF 12140	1	4	4	32	4
<u>Proteus vulgaris</u> X19H ATCC 881	32	128	>128	>128	128
<u>Pseudomonas aeruginosa</u> ATCC 10145	8	16	32	128	16

35

TABLE I (continued)
In vitro activity (MIC microgram/ml)

Test Organisms	Compounds No.			
	46	47	50	51
<u>Staphylococcus aureus</u> TOUR	0.5	0.12	0.12	0.12
<u>Staphylococcus epidermidis</u> ATCC 12228	0.12	0.12	0.008	0.06
<u>Staphylococcus haemolyticus</u> L 602	8	2	0.06	0.12
<u>Streptococcus pyogenes</u> C 203	0.06	0.06	0.06	0.06
<u>Streptococcus pneumoniae</u> UC 41	0.12	0.06	0.12	0.12
<u>Streptococcus faecalis</u> ATCC 7080	0.25	0.12	0.12	0.25
<u>Escherichia coli</u> SKF 12140	>128	>128	1	8
<u>Proteus vulgaris</u> X19H ATCC 881	>128	>128	128	128
<u>Pseudomonas aeruginosa</u> ATCC 10145	>128	>128	8	32

TABLE I (continued)
In vitro activity (MIC microgram/ml)

Test Organisms	Compounds No.			
	52	53	54	55
<u>Staphylococcus aureus</u> TOUR	0.12	0.06	0.5	1
<u>Staphylococcus epidermidis</u> ATCC 12228	0.03	0.06	0.12	0.12
<u>Staphylococcus haemolyticus</u> L 602	0.12	0.06	2	1
<u>Streptococcus pyogenes</u> C 203	0.12	0.06	0.03	0.12
<u>Streptococcus pneumoniae</u> UC 41	0.12	0.12	0.12	0.12
<u>Streptococcus faecalis</u> ATCC 7080	0.12	0.12	2	1
<u>Escherichia coli</u> SKF 12140	32	8	>128	>128
<u>Proteus vulgaris</u> X19H ATCC 881	>128	128	>128	>128
<u>Pseudomonas aeruginosa</u> ATCC 10145	128	32	128	>128

37

The activity against some multi-resistant clinical isolates of Pseudomonas aeruginosa of compounds 22, 23, 25, 26, 27 and 29 is shown in Table II.

TABLE II

<u>Pseudomonas aeruginosa</u> Strain No.	Compound	MIC (microgram/ml)			Compound	Compound
		Compound	Compound	Compound		
L 1138	64	64	32	32	64	64
L 1348	4	4	4	4	4	8
L 1498	64	64	64	64	64	64
L 1533	64	128	64	64	64	128

The activity of the compounds of this invention against Pseudomonas aeruginosa is higher than that of teicoplanin and the closest compounds of European Patent Application Publication No. 218099 and International Patent Application Publication No. WO 88/06600 whose MIC (microgram/ml) against the same microorganism is never lower than 32.

10 The activity of the compounds of the invention against Pseudomonas aeruginosa bacteria is particularly relevant in view of the importance of the infection due to said strain.

15 Clinical infection with P. aeruginosa include local infection, e.g. of wounds (especially burns), the urinary tract, the respiratory tract, the intestine, the eye and the ear, and generalized infections (blood, bone or septicaemic) arising from sites of primary local infection in patients with impaired resistance, and
20 leading to the development of metastatic foci in various organs.

25 The prognosis for patients who develop *Pseudomonas* septicemia is poor and some authors report a very high (sometimes 100%) mortality. See for example "Genetics and Biochemistry of *Pseudomonas*" by P.H. Clarke and M.H. Richmond (Chapter 2), John Wiley and Sons (1975).

30 Furthermore, the teicoplanin compounds of the invention which are different from deglucoteicoplanin and teicoplanin pseudoaglycons show a remarkable higher in vivo activity for what concerns the oral administration with respect to the teicoplanin amide derivatives known in the art.

The ED₅₀ values (mg/kg) of representative compounds of the invention in in vivo tests in mice septicemically infected with Strep. pyogenes C 203, obtained according to the procedure described by V. Arioli et al. (Journal of Antibiotics 29, 511; 1976) are given in table III.

TABLE III - In vivo activities

10	Compound No.	ED ₅₀ (mg/kg)	
		s.c.	p.o.
15	2	0.09	about 60
15	6	0.05	81.2
20	9	0.06	about 80
20	11	0.03	81.2
25	15	0.06	about 60
25	33	0.06	74.4

30 In in vivo Compound 50 was found to be particularly effective in curing mice septicemically infected with E. coli after i.v. administration (40 mg/kg, 7/8 surv./treated) and after s.c. administration (ED₅₀ ≤ 38 mg/kg).

In view of the above reported antimicrobial activity, the compounds of the present invention can be employed as the active ingredients of antimicrobial preparations used in human and veterinary medicine for the prevention and treatment of infective diseases caused by pathogenic bacteria which are susceptible to said active ingredients.

In such treatments, these compounds may be employed as such or in the form of mixtures in any proportion.

The compounds of the present invention can be administered orally, topically or parenterally wherein however, the parenteral administration is preferred.

Depending on the route of administration, these compounds can be formulated into various dosage forms.

Preparations for oral administration may be in the form of capsules, tablets, liquid solutions or suspensions. As known in the art the capsules and tablets may contain excipients such as diluents, e.g. lactose, calcium phosphate, sorbitol and the like, lubricants, binding agents, e.g. magnesium stearate, talc, polyethylene glycol, binding agents, e.g. polyvinylpyrrolidone, gelatin, sorbitol, tragacanth, acacia, flavoring agents, and acceptable disintegrating and wetting agents. The liquid preparations generally in the form of aqueous or oily solutions or suspensions, may contain conventional additives such as suspending agents. For topical use the compounds of the present invention may also be prepared in suitable forms to be applied to the skin, the mucous membranes of the nose and throat or bronchial tissues and may conveniently take the form of creams, ointments, liquid sprays or inhalants, lozenges, or throat paints.

Another advantage of the compounds of the present invention is a marked higher water solubility in a wider

range of pH, and, consequently the normal problems for suitable pharmaceutical composition are avoided.

For medication of the eyes or ears, the preparation
5 may be presented in liquid or semi-liquid form
formulated in hydrophobic or hydrophilic bases as
ointments, creams, lotions, paints, or powders.

For rectal administration the compounds of the
invention are administered in the form of suppositories
10 admixed with conventional vehicles, such as, for
example, cocoa butter, wax, spermaceti or
polyethyleneglycols and their derivatives.

Compositions for injection may take such forms as
suspensions, solutions, or emulsions in oily or aqueous
15 vehicles, and may contain formulatory agents such as
suspending, stabilizing and/or dispersing agents.

Alternatively, the active ingredient may be in
powder form for reconstitution at the time of delivery
with a suitable vehicle, such as sterile water.

20 The amount of active principle to be administered
depends on various factors such as the size and
conditions of the subject to be treated, the route and
frequency of administration, and the causative agent
involved.

25 The compounds of the invention are generally
effective at a dosage comprised between about 0.5 and
about 30 mg of active ingredient per kg of body weight,
preferably divided in 2 to 4 administrations per day.
Particularly desirable compositions are those prepared
30 in the form of dosage units containing from about 20 to
about 300 mg per unit.

The following examples illustrate the manner in which the invention can be practiced but, as such, should not be construed as limiting its overall scope.

5

EXAMPLES - EXPERIMENTAL SECTION

Legenda

10

In the following examples the starting material may be teicoplanin A₂ complex (TGA), a single component thereof or any mixture of two or more of said components.

15

The typical complex mixture essentially consists of five components corresponding to formula I above wherein the aliphatic acyl moieties of the beta-D-2-deoxy-2-aminoglucopyranosyl radical represented by the symbol A are respectively:

20

Z-(4)-decenoyl (AC₁),
8-methylnonanoyl (AC₂),
decanoyle (AC₃),
8-methyldecanoyle (AC₄) and
9-methyldecanoyle (AC₅),

25

B is N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl (AcGlu)

M is alpha-D-mannopyranosyl (Man) and
Y is OH.

30

This mixture is identified by the acronym TGAC₁₋₅. When one of the single components of said mixture is employed as the starting material it is identified as follows: TGAC₁, TGAC₂, TGAC₃, TGAC₄ or TGAC₅, depending on the specific aliphatic acyl rest of the above

35

mentioned aminoglucopyranosyl radical.

When a mixture of one or more components is used it is indicated according to the same system as for the complex. For instance, the acronym TGAC₂₋₅ indicates the mixture of the components 2 to 5 wherein component 1 is no longer present. This mixture is currently obtained when the catalytic hydrogenation saturates the double bond of component 1 transforming it into component 3. The acronym TGAC_{2,3} indicates a mixture of the components 2, 3 and the acronym TGAC_{4,5} indicates a mixture of the components 4 and 5.

The antibiotic L 17392 (i.e. the aglycon of teicoplanin) is represented by the acronym DTG, while the pseugoaglycons L 17054 and L 17046 are represented respectively by the terms TGA3-1 and TGA3-2 and the de-mannosyl pseudoaglycone (European Patent Application Publication No. 301247) by the term DM-TGAC.

The resulting end products in the following tables are identified by reference to formula I above with the indication for the symbol A of the particular aliphatic acyl substituent of the beta-D-2-deoxy-2-aminoglucopyranosyl radical (A/AC) by using the conventional terms AC₁, AC₂, AC₃, AC₄, AC₅ as explained above. When a mixture of two or more components is obtained, this is shown through the same formal system as above.

Examples 1-30

When the N⁶³-carboxyamides of the mixtures TGAC₂₋₅ are desired the following procedures are used:

5

A - Preparation of N¹⁵-benzyloxycarbonyl (CBZ) teicoplanin A₂ complex and single components 1 to 5 thereof

10

A solution of 4.5 ml of benzyl chloroformate in 10 ml of dry acetone is added dropwise at room temperature to a stirred solution of 45 g (about 24 mmol) of 15 teicoplanin A₂ complex (or a single component 1 to 5 thereof) and of 6 ml (about 44 mmol) of triethylamine (TEA) in 300 ml of dimethylformamide (DMF). After about 60 min, 600 ml of ethyl ether is added and the precipitate (about 59 g) is collected by filtration and 20 re-dissolved in 2.5 L of a mixture acetone : water, 1:1 (v/v). The resulting solution is concentrated at 35°C under reduced pressure to a volume of about 1.6 L, then it is extracted with 1.6 L of ethyl ether which is separated and discarded.

25

The aqueous layer is adjusted to pH 4.8 with glacial acetic acid and extracted with 1.5 L of n-butanol. The organic layer is separated, washed with 1.5 L of water (2 x 750 ml), then it is concentrated to a volume of about 200 ml at 45°C under reduced pressure.

30

On adding ethyl acetate (about 800 ml) a solid separates which is collected by filtration, washed with ethyl ether (about 500 ml) and dried at room temperature in vacuo overnight, yielding 45.7 g (about 96%) of pure title compound.

35

B - Preparation of N^{15} -CBZ - teicoplanin A₂ complex and single components 1 to 5 thereof, cyanomethyl ester

5

To a stirred solution of 45 g (about 22 mmol) of N^{15} -CBZ-teicoplanin A₂ complex (or a single component thereof) in 450 ml of DMF, 5.25 l (about 37 mmol) of TEA and 60 ml of chloroacetonitrile are added at room temperature. After 20 h, the reaction mixture is poured into 4.5 L of ethyl acetate and the precipitate (about 50 g) is collected by filtration and re-dissolved in 900 ml of a mixture methanol : water 1:1 (v/v). The resulting solution is adjusted to pH 5.5 with glacial acetic acid, then 1.1 L of n-butanol is added. Most of the methanol is evaporated at 35°C under reduced pressure to obtain a mixture (about 1.5 L) of n-butanol and water from which the organic layer is separated, washed with 500 ml of water and concentrated at 40°C under reduced pressure to a volume of about 200 ml. On adding 800 ml of ethyl acetate a solid separates which is collected, washed with 500 ml of ethyl ether and dried at 35°C in vacuo overnight to give 44.2 g (about 98% yield) of pure title compound.

25

C - Preparation of N^{63} -carboxyamides of N^{15} -CBZ-teicoplanin A₂ complex and single components 1 to 5 thereof

30

A solution of 16 g (about 8 mmol) of N^{15} -CBZ-teicoplanin A₂ complex (or a single component 1 to 5 thereof), cyanomethyl ester and of a large excess (from 35 50 to 100 mmol) of the proper amine reactant in 160 ml

of DMF or DMSO is stirred at room temperature for 60-120 min, afterwards 160 ml of absolute ethanol is added followed by 1.5 L of ethyl acetate. A solid separates which is collected by filtration and washed with 500 ml 5 of ethyl ether, then it is dried at room temperature in the air to obtain a powder (yields generally > 85%) which is enough pure (HPLC titre generally > 90%) for the next hydrogenation step.

10

D - Preparation of N⁶³-carboxyamides of teicoplanin A₂ complex and single components 2 to 5 thereof

15

The product obtained as described above (5 mmol) is dissolved in 500 ml of a mixture methanol : 0.04 N hydrochloric acid 7/3 (v/v) and the resulting solution is hydrogenated at room temperature and pressure in the 20 presence of 5% Pd/C (5 g). As soon as the reaction is completed (HPLC) the catalyst is removed by filtration through a panel of celite (BDH 545). The clear filtrate is adjusted to pH 6.5 with 1 N NaOH and 500 ml of n-butanol is added. The resulting mixture is 25 concentrated, at 40°C under reduced pressure, to a volume of about 150 ml, then 350 ml of ethyl ether is added and the precipitate is collected by filtration. When the reaction is carried out in a substrate containing a derivative corresponding to the component 1 30 of teicoplanin A₂ complex, the relative end product does not contain the component 1 carboxyamide since it is almost completely transformed into component 3 carboxyamide.

35

E - Purification of the products by reverse phase column chromatography

5 Crude products obtained as described above (10 g) are dissolved in a mixture (300 ml) acetonitrile : water 1:1 (v/v). Water is then added until a cloudy solution forms (in any case no more than 700 ml of water is added) which is loaded at the top of a column of 500 g
10 of silanized Silica-gel (0.06-0.2 mm; Merck Co.) prepared in the same solvent mixture (i.e., CH₃CN and H₂O in the ratio calculated on the base of the amount of H₂O added to obtain the above cloudy solution) as obtained at the beginning of the precipitation. The
15 column is developed with a linear gradient, from 10% to 80% of acetonitrile in water previously adjusted to pH 3.2 with glacial acetic acid, in 15 h at the rate of 400 ml/h while collecting 25 ml fractions which are monitored by HPLC. Those fractions containing the
20 desired pure product are combined and enough n-butanol is added to obtain, after concentration at 45°C under vacuum a cloudy dry butanolic solution. On adding three volumes, of ethyl ether a solid separates which is collected, washed with ethyl ether and dried at room
25 temperature in vacuo overnight to give pure final compound.

The compounds of the invention (Table IV) are thus obtained as the free bases (FB) when the only basic function present in the molecule is the free amino group
30 in the position 15 of teicoplanin A₂ complex, or when the additional amino group introduced with the amide substituent is not enough basic to form an acid addition salt with acetic acid. Otherwise they are recovered as the acetates.

Preparation of the corresponding hydrochlorides is carried out, when this acid addition salt form is required, according to the following procedures:

1 mmol of an amide of teicoplanin A₂ complex (or a single component thereof), either as the free base or as the acetate, is dissolved in 10 ml of DMF. A 10% molar excess of 10 N HCl (0.11 ml for one amino function to be salified, 0.22 ml for two amino groups, etc.,) is then added under stirring at 5°C, afterwards 40 ml of ethyl ether is added. The precipitate which forms is then collected by filtration, washed with ethyl ether and dried at room temperature in vacuo overnight (yields > 95%).

15

When the N⁶³-carboxyamides of component 1 (TGAC₁) of teicoplanin A₂ complex (or the mixture TGAC₁₋₅) are desired, the following procedures are used:

20

A' - Preparation of N¹⁵-tert-butyloxycarbonyl (t-BOC) teicoplanin A₂ complex and single components 1 to 5 thereof

25

A solution of 10 g (about 5 mmol) of teicoplanin A₂ complex or a single component 1 to 5 thereof, 1.2 ml (about 8.5 mmol) of triethylamine (TEA) and 2.4 g (about 8 mmol) of tert-butyl-2,4,5-trichlorophenylcarbamate in 100 ml of dimethylformamide (DMF) is stirred at room temperature for 24 h. Then it is poured into 200 ml of water. The resulting cloudy solution is adjusted at pH 3 with 1N HCl and it is extracted with 600 ml of a mixture n-butanol/ethyl acetate 35:65 (v/v). The organic layer is separated, washed with water (2 x 100 ml), then it is concentrated to a volume of about 100 ml at 45°C

under reduced pressure. On adding ethyl acetate (about 400 ml) a solid separates which is collected by filtration, washed with ethyl ether (about 200 ml) and dried at room temperature in vacuo overnight, yielding
5 10.3 g (about 98%) of pure title compound.

B' - Preparation of N¹⁵-t-BOC-teicoplanin A₂ complex and a single component 1 to 5 thereof,
10 cyanomethyl ester

Substantially following the above procedure B, the title compound was obtained (about 98% yield) from t-BOC-teicoplanin A₂ complex.
15

C' - Preparation of N⁶³-carboxyamides of N¹⁵-t-BOC-teicoplanin A₂ complex and a single component
20 1 to 5 thereof

Substantially following the above procedure C, but using dimethylsulfoxide (DMSO) instead of DMF as the preferred solvent, the title compounds were obtained
25 from N¹⁵-BOC-teicoplanin A₂ complex, cyanomethyl ester, substantially with the same yields (generally >85%) and purity (HPLC titre generally >90%).

D' - Preparation of N⁶³-carboxyamides of
teicoplanin A₂ complex or a single component thereof

The product (N⁶³-carboxyamide of
5 N¹⁵-t-BOC-teicoplanin A₂ complex or a single component
thereof) (4 mmol) is dissolved in 40 ml of dry
trifluoroacetic acid (TFA) at 10°C. As soon as a clear
solution is formed (about 2 min) (in any case no more
than 5 min after the addition of TFA), the reaction
10 mixture is diluted with 40 ml of methanol while cooling
at 10°C. On adding 420 ml of ethyl ether a precipitate
separates which is collected by filtration and washed
with ethyl ether (5 x 200 ml).

Purification of the products is readily carried out
15 by dissolving the crude product (5 g) in a mixture
(150 ml) acetonitrile/water, 1:1, (v/v), adjusting the
resulting solution at pH 6 with 1N NaOH and after
diluting with water by following the same
chromatographic procedure as described above (E).

20

When the N⁶³-carboxyamides of deglucoteicoplanin
(DTG) are desired the following procedures are used:

25

A" - Preparation of N¹⁵-tert-butyloxycarbonyl
(t-Boc) deglucoteicoplanin

To a stirred solution of 45 g (about 37 mmol) of
30 antibiotic L 17392 (deglucoteicoplanin) in 600 ml of
DMF, 19.3 g (about 65 mmol) of tert-butyl-2,4,5-tri-
chlorophenylcarbonate and 10.2 ml (about 74 mmol) of TEA
are added. The reaction mixture is stirred at room
temperature for 24 h afterwards it is pured into 1.5 L
35 of water. The resulting solution is adjusted to pH 3

with 1 N hydrochloric acid, then it is extracted with 3 L of a mixture ethyl acetate : n-butanol 2:1 (v/v). The organic layer is separated, washed with 1 L of water, then it is concentrated at 40°C under vacuum to a 5 volume of about 300 ml. On adding 700 ml of ethyl ether, a solid separates which is collected by filtration, washed with 200 ml of ethyl ether and dried at room temperature in vacuo overnight, yielding 44 g (92%) of pure title compound.

10

B" - Preparation of N^{15} -t-BOC-deglucoteicoplanin cyanomethyl ester

15

A solution of 44 g (about 33 mmol) of N^{15} -t-BOC deglucoteicoplanin, 4.7 ml (about 34 mmol) of TEA and 44 ml of chloroacetonitrile in 440 ml of DMF is stirred at room temperature for 20 h, afterwards 1 L of ethyl 20 acetate is added and the precipitate is collected by filtration. It is re-dissolved (about 46 g) in 1.5 L of a mixture methanol : water 1:2 (v/v) and the resulting solution is adjusted to pH 5.6 with glacial acetic acid.

25 After adding 2 L of n-butanol, the most methanol is evaporated at 30°C under vacuum and the organic layer is separated, washed with 1 L of water, then it is concentrated at 35°C under vacuum to a final volume of about 300 ml. On adding 700 ml of ethyl ether, a solid 30 separates which is collected by filtration, washed with 500 ml of ethyl ether, then it is dried at room temperature in vacuo overnight to give 42.5 g (96%) of pure title compound.

35

C" - Preparation of N⁶³-carboxyamides of N¹⁵-t-BOC
deglucoteicoplanin

To a stirred solution of 14 g (about 10 mmol) of
5 N¹⁵-t-BOC-deglucoteicoplanin and of a large excess (from
100 to 150 mmol) of the proper reactant amine in 200 ml
of DMF, 8.9 ml (about 150 mmol) of glacial acetic acid
is added at room temperature. (The molar amount of
10 glacial acetic acid depends on the structure of the
reactant amine. In fact, for one mmol of amine, 0.5 mmol
of glacial acetic acid is required when the amine does
not contain additional basic functions, 1 mmol of
glacial acetic acid when the amine contain one
15 additional basic function, 2 mmol when the amine contain
two additional basic functions, etc. Although the
presence of acetic acid is unnecessary for the
condensation, it is sometimes suitable to avoid side
epimerization of the molecule at the C₃ position which
might occur under basic conditions.

20 Furthermore, the presence of the acid does not
influence the rate of the condensation reaction in the
majority of cases.

After 3-6 h (the reaction, except a few cases, is
25 in general completed within 3 h), 600 ml of ethyl
acetate is added and the precipitate is collected by
filtration, washed with 200 ml of ethyl ether and dried
at room temperature in vacuo overnight to give a product
which is enough pure for the next deprotection step
(yields > 75%).

30

D" - Preparation of N⁶³-carboxyamides of
deglucoteicoplanin

5

A solution of 1 mmol of product obtained as described above, which has in general a HPLC titre > 85% and contain some acetate of the reactant amine as the main impurity, in 25-30 ml of anhydrous trifluoroacetic acid (TFA) is stirred at room temperature for 20 min, then solvent is evaporated at 25°C under reduced pressure. The oily residue is re-dissolved in 50 ml of a mixture water : acetonitrile 6:4 (v/v) and the resulting solution is diluted with water until precipitation starts. The suspension thus obtained is adjusted to pH 3.0 with 1N hydrochloric acid (if necessary) and the resulting solution is loaded at the top of a column of 100 g of silanized Silica-gel (0.06-0.2 min; Merck Co.) in water.

20

E" - Purification of the products by reverse phase column chromatography

25

The column loaded with the product, as described above, is developed with 1 L of water, then elution is performed with a linear gradient from 10% of acetonitrile in water to 50% of acetonitrile in 0.01 N hydrochloric acid, in 15 h at the rate of 200 ml/h, while collecting 10 ml fractions. Those fractions containing pure product are pooled and enough n-butanol is added to obtain, after concentration of the resulting mixture, a cloudy dry butanolic solution (30-100 ml). On adding three volumes of ethyl ether, a solid separates

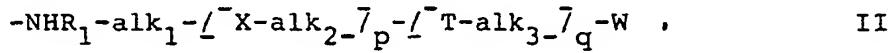
which is collected by filtration, washed with ethyl ether and dried at room temperature in vacuo for 2-3 days to yield pure final amides of deglucoteicoplanin as the hydrochlorides.

5 The corresponding trifluoroacetates are obtained by following the above chromatographic procedure of purification but eluting with a linear gradient from 10% to 60% of acetonitrile in water and maintaining pH of the eluent at 2.5 adding trifluoroacetic acid.

10

By using the appropriate reagents TGAC, a single component thereof, DTG or DMTGAC and an amine of formula

15



under the conditions described above the compounds
20 represented in Table IV, are obtained.

TABLE IV:
TGA, DMTRGAC and DTG carboxyamide derivatives

Comp. Teicoplanin No. staring material	Reagents	Amine material	End product of formula I (R = H)				
			A/AC	B	M	Y	
1	TGAC ₂₋₅	NH ₂ (CH ₂) ₂ -NH(CH ₂) ₂ -NH ₂		AC ₂₋₅	AcGlu	Man	-NH(CH ₂) ₂ -NH(CH ₂) ₂ -NH ₂
2	TGAC ₂	NH ₂ (CH ₂) ₂ -NH(CH ₂) ₂ -NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₂ -NH(CH ₂) ₂ -NH ₂
3	TGAC ₂₋₅	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₂ -NH ₂		AC ₂₋₅	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₂ -NH ₂
4	TGAC ₂	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₃ -NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₃ -NH ₂
5	TGAC ₂₋₅	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₄ -NH ₂		AC ₂₋₅	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₄ NH ₂ *
6	TGAC ₂	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₄ -NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₄ NH ₂ *
7	TGAC ₂	NH ₂ (CH ₂) ₂ -NH(CH ₂) ₃ NH(CH ₂) ₂ NH ₂	AC ₂	AcGlu	Man	-NH(CH ₂) ₂ -NH(CH ₂) ₃ NH(CH ₂) ₂ NH ₂	

* in admixture with -NH(CH₂)₄NH(CH₂)₃-NH₂

TABLE IV (continued)

Comp. No.	Teicoplanin starting material	Reagents			End product of formula I (R = H)			
		Amine			A/AC	B	M	Y
8	TGAC ₂	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₂ -NH(CH ₂) ₃ NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₂ -NH(CH ₂) ₃ NH ₂	
9	TGAC ₂	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₃ NH(CH ₂) ₃ -NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₃ NH(CH ₂) ₃ -NH ₂	
10	TGAC ₁₋₅	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₄ NH(CH ₂) ₃ -NH ₂		AC ₂₋₅	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₄ NH(CH ₂) ₃ -NH ₂	
11	TGAC ₂	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₄ NH(CH ₂) ₃ -NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₄ NH(CH ₂) ₃ -NH ₂	
12	TGAC ₂₋₅	NH ₂ (CH ₂) ₂ -NH(CH ₂) ₂ NH(CH ₂) ₂ -NH ₂		AC ₂₋₅	AcGlu	Man	-NH(CH ₂) ₂ -NH(CH ₂) ₂ NH(CH ₂) ₂ -NH ₂	
13	TGAC ₂	NH ₂ (CH ₂) ₂ -NH(CH ₂) ₂ NH(CH ₂) ₂ -NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₂ -NH(CH ₂) ₂ NH(CH ₂) ₂ -NH ₂	
14	TGAC ₂	NH ₂ (CH ₂) ₂ -N/[CH ₂) ₂ NH ₂ -7 ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₂ -N/[CH ₂) ₂ NH ₂ -7 ₂	
15	TGAC ₂	NH ₂ (CH ₂) ₃ -N[] ₂ N-(CH ₂) ₃ -NH ₂		AC ₂	AcGlu	Man	-NH(CH ₂) ₃ -N[] ₂ N-(CH ₂) ₃ -NH ₂	

TABLE IV (continued)

Comp. No.	Teicoplanin starting material	Reagents	End product of formula I (R = H)		
			Amine	A/AC	B M
16	TGAC ₂	NH ₂ (CH ₂) ₂ -NH(CH ₂) ₂ -NH(CH ₂) ₂ NH ₂	AC ₂	AcGlu Man	-NH(CH ₂) ₂ -NH(CH ₂) ₂ -NH(CH ₂) ₂ NH ₂
17	TGAC ₂	NH ₂ -CH(CH ₃)CH ₂ -O-CH ₂ CH(CH ₃) ₂ -NH ₂	AC ₂	AcGlu Man	-NH-CH(CH ₃)CH ₂ -O-CH ₂ CH(CH ₃) ₂ -NH ₂ * 55
18	TGAC ₂	NH ₂ -CH(CH ₃)CH ₂ -O-(CH ₂) ₂ -NH ₂	AC ₂	AcGlu Man	-NH-CH(CH ₃)CH ₂ -O-(CH ₂) ₂ -NH ₂
19	TGAC ₂	NH ₂ -N(CH ₂) ₃ -N(CH ₃) ₂	AC ₂	AcGlu Man	-N 
20	DM-TGAC ₂	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₄ -NH(CH ₂) ₃ -NH ₂	AC ₂	AcGlu	H
					-NH(CH ₂) ₃ -NH(CH ₂) ₄ -NH(CH ₂) ₃ -NH ₂

* in admixture with -NHCH(CH₃)CH₂-OCH(CH₃)CH₂-OCH₂(CH₃)NH₂

TABLE IV (continued)

Comp. No.	Teicoplanin starting material	Reagents			End product of formula I (R = H)		
		Amine	A/AC	B	M	Y	
21	DTG	NH ₂ (CH ₂) ₂ NH(CH ₂) ₂ -NH ₂	H	H	H	-NH(CH ₂) ₂ NH(CH ₂) ₂ -NH ₂	
22	DTG	NH ₂ (CH ₂) ₃ NH(CH ₂) ₃ -NH ₂	H	H	H	-NH(CH ₂) ₃ NH(CH ₂) ₃ -NH ₂	
23	DTG	NH ₂ (CH ₂) ₃ NH(CH ₂) ₄ NH ₂	H	H	H	-NH(CH ₂) ₃ NH(CH ₂) ₄ -NH ₂	*
24	DTG	NH ₂ (CH ₂) ₂ NH(CH ₂) ₃ NH(CH ₂) ₂ NH ₂	H	H	H	-NH(CH ₂) ₂ NH(CH ₂) ₃ NH(CH ₂) ₂ -NH ₂	
25	DTG	NH ₂ (CH ₂) ₃ NH(CH ₂) ₂ NH(CH ₂) ₃ -NH ₂	H	H	H	-NH(CH ₂) ₃ NH(CH ₂) ₂ NH(CH ₂) ₃ -NH ₂	
26	DTG	NH ₂ (CH ₂) ₃ NH(CH ₂) ₃ NH(CH ₂) ₃ -NH ₂	H	H	H	-NH(CH ₂) ₃ NH(CH ₂) ₃ NH(CH ₂) ₃ -NH ₂	
27	DTG	NH ₂ (CH ₂) ₃ NH(CH ₂) ₄ NH(CH ₂) ₃ -NH ₂	H	H	H	-NH(CH ₂) ₃ NH(CH ₂) ₄ NH(CH ₂) ₃ -NH ₂	

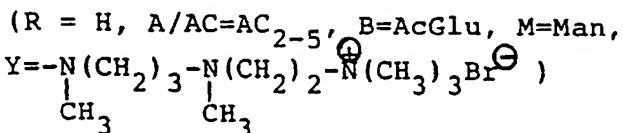
* in admixture with -NH(CH₂)₄NH(CH₂)₃-NH₂

TABLE IV (continued)

Comp. Teicoplanin No. starting material	Reagents			End product of formula I (R = H)			
	Amine	A/AC	B	M	Y		
28	DTG	NH ₂ (CH ₂) ₂ NH(CH ₂) ₂ NH ₂	H	H	H	-NH(CH ₂) ₂ NH(CH ₂) ₂ NH(CH ₂) ₂ -NH ₂	60
29	DTG	NH ₂ (CH ₂) ₂ N(CH ₂) ₂ NH ₂ - ⁷ 2	H	H	H	-NH(CH ₂) ₂ N(CH ₂) ₂ NH ₂ - ⁷ 2	
30	DTG	NH ₂ (CH ₂) ₃ -N(cyclohexyl) ₂ -N(CH ₂) ₃ -NH ₂	H	H	H	-NH(CH ₂) ₃ -N(cyclohexyl) ₂ -N(CH ₂) ₃ -NH ₂	

Example 31

Preparation of the compound 31 of formula I



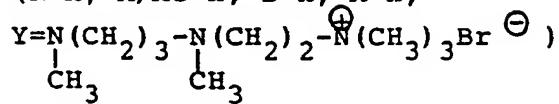
A solution of 2 g (about 1 mmol) of N¹⁵-CBZ-tei-coplanin A₂ complex, cyanomethyl ester, prepared as
 10 described above and 2 ml of 1,3-dimethyl-1,3-propane-diamine in 20 ml of DMF is stirred at room temperature for 2 h, afterwards 20 ml of absolute ethanol is added followed by 200 ml of ethyl acetate. A solid separates which is collected by filtration, washed with 50 ml of
 15 ethyl ether and dried in vacuo at room temperature overnight, to yield 1.95 g of pure N¹⁵-CBZ-teicoplanin A₂ complex, 1-methyl-3-(methylamino)propyl-amide.

To a stirred solution of 1.37 g (0.65 mmol) of the above compound in 100 ml of dry methanol, 1 g (9.4 mmol)
 20 of anhydrous sodium bicarbonate and 2.5 g (10.1 mmol) of 2-bromoethyl trimethylammonium bromide are added at room temperature. The reaction mixture is stirred at 45°C for three days, then it is cooled to 10°C and poured into 100 ml of water. Methanol is evaporated at 30°C under
 25 reduced pressure and the aqueous phase is extracted with 300 ml of a mixture n-BuOH/EtOAc 1/2 (v/v). The organic layer is separated and concentrated at 40°C under reduced pressure to a small volume (about 20 ml). On adding 180 ml of ethyl ether, the precipitated solid
 30 (1.12 g of N¹⁵-CBZ precursor of the title compound) is collected and hydrogenated under the same conditions described in Example 1, to give 0.45 g of compound 31.

Example 32

Preparation of the compound 32 of formula I

(R=H, A/AC=H, B=H, M=H,

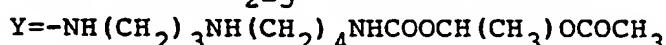


Following the same procedure described in Example
 31 above but using a solution of 2 g of
 10 $N^{15}\text{-CBZ-deglucotetraicoplanin, cyanomethyl ester, the}$
 compound 32 is obtained.

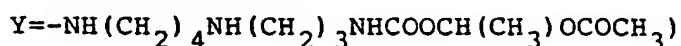
15 Example 33

Preparation of the compound 33 of formula I

(R=H, A/AC=AC₂₋₅, B=AcGlu, M=Man,



in admixture with



To a stirred solution of 2 g (0.9 mmol) of $N^{15}\text{-CBZ}$
 25 derivative of compound 5 (prepared as described in
 Example 1 above), in 50 ml of dry DMF, 1.2 g (11 mmol)
 of anhydrous sodium carbonate and 2.7 g (10 mmol) of
 alpha-acetoxy-ethyl para-nitrophenyl carbonate are added
 at room temperature. After 3 h, the reaction mixture is
 30 poured into 500 ml of ethyl acetate and the precipitated
 solid is collected, washed with 100 ml of ethyl acetate
 and hydrogenated as described in Example 1 above, to
 yield 0.57 g of the title compound 33.

Example 34

Preparation of the compound 34 of formula I

(R=H, A/AC=H, B=H, M=H,

5 Y=NH(CH₂)₃NH(CH₂)₄NHCOOCH(CH₃)OCOCH₃
in admixture with

Y=NH(CH₂)₄NH(CH₂)₃NHCOOCH(CH₃)OCOCH₃)

10 Following the procedure as described in Example 32
but using 2 g of N¹⁵-CBZ derivative of compound 23,
0.6 g of compound 34 are prepared.

15

Examples 35-36

Preparation of compound 35 of formula I

(R=H, A/AC=AC₂₋₅, B=AcGlu, M=Man,

20 Y=-N(CH₂)₃-¹N-CH₂CH₂(OCH₂CH₂)₂OH
 | |
 CH₃ CH₃

and of compound 36 of formula I

(R=H, A/AC=AC₂₋₅, B=AcGlu, M=Man,

25 Y=-N-(CH₂)₃-¹NCH₂CH₂OCH₂CH₂OH
 | |
 CH₃ CH₃

To a stirred suspension of 5.3 g (about 2.5 mmol) of N¹⁵-CBZ-teicoplanin A₂ in 560 ml of methanol, 1-methyl-3-(methylamino)propyl-amide (prepared as described above in Example 31), 17 ml of the proper chloroethoxy-hydroxyethyl reagent of formula ClCH₂CH₂(OCH₂CH₂)₂OH and ClCH₂CH₂OCH₂CH₂OH respectively and 1.86 g (13.5 mmol) of potassium carbonate are added at room temperature. After stirring at 45°C for 3 h, the reaction mixture is cooled to 15°C and adjusted at pH 6

with 1N HCl. Methanol is evaporated at 30°C under reduced pressure, and the solid residue is hydrogenated as described in Example 1 to yield 1.9 g of compound 35 or 0.97 g of compound 36.

5

Examples 37-41

10 Preparation of TGA3-1 amide derivatives

A solution of 4 g (about 2 mmol) of the proper amide derivative of a teicoplanin A₂ complex or a single component thereof prepared as described above and reported in table V below, in 100 ml of 90% aqueous trifluoroacetic acid is stirred at room temperature for 2h; afterwards the solvents are evaporated and the oily residue is re-dissolved in 200 ml of H₂O. After 15 adjusting at pH 8, the resulting solution is loaded on a column of 400 g of silanized silicagel in H₂O. Chromatography is performed as described above in 20 Example 1 to give the title compounds.

25

TABLE V

TGA3-1 carboxyamide derivatives

No.	Compound No.	Reagent	End product of formula I			
			A/AC	B	M	Y
37	1	H	AcGlu	Man	-NH(CH ₂) ₂ NH(CH ₂) ₂ NH ₂	
38	5	H	AcGlu	Man	-NH(CH ₂) ₃ -NH(CH ₂) ₄ -NH ₂	*
39	10	H	AcGlu	Man	-NH(CH ₂) ₃ NH(CH ₂) ₄ NH(CH ₂) ₃ -NH ₂	
40	12	H	AcGlu	Man	-NH(CH ₂) ₂ NH(CH ₂) ₂ NH(CH ₂) ₂ -NH ₂	
41	14	H	AcGlu	Man	-NH(CH ₂) ₂ N/(CH ₂) ₂ NH ₂ -7 ₂	

(*) in admixture with -NH(CH₂)₄-NH(CH₂)₃-NH₂

Examples 42-45

Preparation of TGA3-2 amide derivatives

5

A suspension of 4 g (about 2 mmol) of the proper amide derivative of a teicoplanin compound prepared as described above and reported in table VI below, in 80 ml of 1,2-dimethoxyethane (DME) is stirred at room temperature for 2 days, while bubbling dry HCl, afterwards the insoluble matter is collected by filtration. Purification by column chromatography as described above (Example 1) yields the title compounds.

10
15

67

TABLE V

TGA3-2 carboxyamide derivatives

No.	Compound No.	Reagent	End product of formula I		
			A/AC	B	M
42	1	H	AcGlu	H	-NH(CH ₂) ₂ -NH(CH ₂) ₂ NH ₂
43	5	H	AcGlu	H	-NH(CH ₂) ₃ -NH(CH ₂) ₄ -NH ₂ *
44	10	H	AcGlu	H	-NH(CH ₂) ₃ NH(CH ₂) ₄ -NH(CH ₂) ₃ -NH ₂
45	12	H	AcGlu	H	-NH(CH ₂) ₂ -NH(CH ₂) ₂ -NH(CH ₂) ₂ -NH ₂

(*) in admixture with -NH(CH₂)₄-NH(CH₂)₃-NH₂

Examples 46-55A''' - General Procedure (by employing
diphenyl phosphorazide)

5

To a stirred solution of 6 mmol of teicoplanin A2, or a single component thereof (or a mixture of its components in any proportion), or N¹⁵-t-BOC-deglucoteicoplanin carbonyl (t-BOC) deglucoteicoplanin in 60 mL of dimethylsulfoxide (DMSO), 30 mmol of the appropriate intermediate amine (prepared as described below) and 10 mmol of diphenyl phosphorazidate (DPPA) are added at 0-5°C. After stirring at room temperature overnight, 240 mL of ethyl acetate is added and the precipitated solid is collected and purified by reverse phase column chromatography as described previously (Method E), thus obtaining pure TGAC amides or N¹⁵-t-BOC-deglucoteicoplanin amides (BOC-DTG amides).

10

In the case of BOC-DTG amides, or amides containing BOC-protecting groups on the amidic portion, the BOC-protecting groups are removed by dissolving 1 mmol of these compounds in 30 mL of anhydrous trifluoroacetic acid at room temperature and following the same procedure as described previously (e.g., Method D" for the preparation of the N⁶³-carboxyamides of DTG).

15

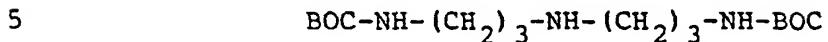
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B''' - Preparation of the intermediate amines
of compounds 46-55

25

1. Diamine O,O'-bis(2-aminopropyl)polyethylene glycol 1900 (JeffamineTM ED 2001) was purchased from Fluka Chemie AG (intermediate amine of compound 46)

2. For the intermediate reacting amines of compounds 47-52 a common intermediate di-(3-BOC-aminopropyl)amine



has been preliminarily prepared as follows:

10 A solution of 142 g of 2-(tert-butoxycarbonyloxy-imino-2-phenylacetonitrile (BOC-ON, Aldrich-Chemie) in 300 mL of tetrahydrofuran (THF) is added dropwise at 10°C to a stirred solution of 42 mL of bis-(3-amino-propyl)amine (Fluka Chemie AG) in 400 mL of THF. After
15 16 hours at room temperature, the solvent is evaporated and the oily residue is dissolved in 1 liter of ethyl acetate. The resulting solution is washed with 1N NaOH (200 mL), and then with water (2 x 300 mL); afterwards it is extracted with 0.01N HCl (2 x 500 mL). The aqueous
20 phase is adjusted at pH 8 with 1N NaOH and extracted with 500 mL of n-butanol. The organic layer is separated, washed with 250 mL of water, and then it is concentrated to a final volume of about 70 mL. On standing at 6°C overnight crystals form which are
25 collected by filtration, to give 75 g of pure title compound, as the free base.

$^1\text{H NMR: } 2.93, 2.44, 1.47 (\text{CH}_2); 1.38 (\text{N-BOC}) 6.68 (\text{NH}).$

30

3. N',N"-di-t-BOC-Tris-(3-aminopropyl)amine (for derivatives 47-50):

To a stirred solution of 45 g of the above di-t-BOC intermediate triamine in 500 mL of absolute ethanol, 21 mL of 3-bromo-propionitrile and 25 g of potassium carbonate are added at room temperature. The reaction 5 mixture is stirred overnight, then it is filtered and concentrated to a final volume of about 100 mL, afterwards it is diluted with 800 mL of water. The resulting solution (pH 8) is extracted with ethyl acetate (2 x 800 mL). The organic layer is separated and 10 washed with water (2 x 200 mL), afterwards it is concentrated to a final volume of about 100 mL. On standing at 6°C overnight, a crystalline solid separates which is collected by filtration, yielding 34 g of di-(3-t-BOC-aminopropyl)amino-1-propionitrile.

15 ¹H NMR: 2.94, 2.63, 2.54, 2.37, 1.47 (CH₂); 1.36 (N-BOC); 6.73 (NH).

This product is dissolved in 200 mL of an ethanolic 20 solution containing 8.5 g of NaOH. To the resulting solution, 4 g of Raney nickel, active catalyst (Aldrich-Chemie), is added and the suspension is hydrogenated at 2.5 atm for 10 hours. The catalyst is filtered off and the solvent is evaporated. The oily residue is dissolved in 500 mL of ethyl acetate and the 25 resulting solution is washed with water (2 x 100 mL), afterwards the organic solvent is evaporated to give about 34 g of the title compound.

1H NMR: 2.93, 2.54, 2.32, 1.49 (CH₂), 1.41 (N-BOC); 6.77 (NH).

4. 3-(3-aminopropyl)-3-(3,3-dimethylaminopropyl)-amino-1-propylamine (for derivative 51):

To a stirred solution of 19 g of 3,3-dimethyl-amino-1-propyl chloride, hydrochloride in 400 mL of absolute ethanol, 20 g of the di-t-BOC intermediate triamine and 28 g of potassium carbonate are added at 5 room temperature followed by 3 g of potassium iodide. The reaction mixture is refluxed for 6 hours, then it is filtered, and the solvent is evaporated. The residue is re-dissolved in 400 mL of water and the resulting solution is extracted with 600 mL of ethyl acetate. The 10 organic layer is separated, washed with water (2 x 200 mL), and then the solvent is evaporated, yielding an oily residue (8.7 g), the di-t-BOC derivative of the title compound, enough pure for the next step.

15 ^1H NMR: 2.91, 2.42, 2.31, 2.16, 1.47 (CH_2), 1.36 (N-BOC), 2.09 (NCH₃).

A solution of this product in 30 mL of methylene chloride is treated with 30 mL of dry trifluoroacetic acid at room temperature for 2 hours, afterwards the 20 solvents are evaporated. The oily residue is dissolved in 40 mL of absolute ethanol and dry HCl is bubbled at room temperature until complete precipitation of the product is observed. After filtration, 3.8 g of the title compound are obtained, as the tetra-hydrochloride.

25 ^1H NMR: 3.2-2.91 (6-CH₂); 2.13-1.90 (3-CH₂); 2.73 (NCH₃).

For the condensation with BOC-DTG, the free base is used which is prepared by dissolving the tetra-hydro-30 chloride (10 mmol) in 1N NaOH (40 mL), followed by evaporation of the resulting solution to dryness. The residue is then suspended in methylene chloride (100 mL) and the insoluble matter is filtered off. The solvent is evaporated and the oily residue is used as such without 35 further purification.

5. 3-(3-aminopropyl)-3-(2,2-diethylaminoethyl)-
-amino-1-propylamine (for derivative 52):

By following exactly the same procedure as that
5 described above, but using 2,2-diethylamino-1-ethyl
chloride, hydrochloride (21 g) for the reaction with the
di-t-BOC intermediate triamine (20 g), 11 g of the
di-t-BOC derivative of the title compound are first
obtained. The protecting groups are then removed
10 analogously by treatment with trifluoroacetic acid in
methylene chloride solution. The free base (as oil) is
finally obtained as described above, yielding the title
compound (8.2 g).

¹H NMR: 2.6-2.3 (8-CH₂) ; 1.42 (2-CH₂) ; 0.92 (2-CH₃).
15

The course of these reactions and the homogeneity
of the final polyamines are checked by TLC on silica gel
60 F₂₅₄ pre-coated plates (Merck Co.), using a methylene
chloride/methanol 9:1 (v/v) mixture containing 1%
20 ammonium hydroxide as the mobile phase. The spots are
developed with iodine.

25 6. 4-(3,3-dimethylaminopropyl)piperazine
 (for derivative 53):

To a stirred solution of 15.8 g of 3,3-dimethyl-
amino-1-propyl chloride in 300 mL of absolute ethanol, 9
30 mL of 1-benzyl-piperazine and 14 g of potassium
carbonate are added. The reaction mixture is stirred
under reflux for 6 hours, afterwards it is cooled at
room temperature and filtered. Solvent is evaporated and
the oily residue is dissolved in 300 mL of water. The
35 resulting solution is extracted with methylene chloride

(2 x 200 mL). The organic layer is separated, washed with 200 mL of water and then solvent is evaporated. The oily residue (9 g) is dissolved in 300 mL of 95% ethanol and hydrogenated (25°C, 1 atm) over 3 g of 10% Pd/C.

5 About 1 L of H₂ is absorbed within 6 hours. The catalyst is filtered off and dry HCl is bubbled into the clear filtrate. A solid separated which is collected, washed with absolute ethanol and dried in vacuo at room temperature overnight, to give 7 g of pure title

10 compound, as tri-hydrochloride.

¹H NMR: 2.79, 2.53, 2.30 (CH₂-piperazine) 2.79, 2.23, 2.15 (CH₂ dimethylaminopropyl); 2.10 (NCH₃).

The free base is obtained by dissolving this
15 tri-hydrochloride (6 g) in 2N NaOH (30 mL) followed by extraction with methylene chloride (170 mL) and evaporation of the organic solvent. The resulting oily residue is used without further purification in the preparation of compound 53.

20

7. N,N'-Bis(3-aminopropyl)nonane-1,5-diamine and
N,N'-Bis(3-aminopropyl)decane-1,5-diamine

25

These derivatives are known compounds and are prepared according to the method of Israel, M.J., Rosenfield S.S., Modest, E.J., J. Med. Chem. 1964, 7, 710 by mono- and dy-cyanoethylation of the appropriate
30 alpha-,omega-alkylenediamines, followed by catalytic reduction of the nitriles under unusually mild conditions.

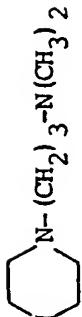
For compounds prepared according to the procedure
35 of A." (46-55), see Table VI.

TABLE VI:
TGA and DTG carboxyamide derivatives

Comp. Teicoplanin No. starting material	Reagents	Amine	End product of formula I (R = H)				Y
			A/AC	B	M	AcGlu	
46 TGAC ₂	NH ₂ -(CH) (CH ₃)CH ₂ -OCCH ₂ CH ₂ -7-4 ₂ OCH ₂ CH(NH ₂)NH ₂	AC ₂				-NH-CH(CH ₃)CH ₂ -OCCH ₂ CH ₂ -7-4 ₂ O-CH ₂ CH(NH ₂)NH ₂	
47 TGAC ₁₋₅	NH ₂ (CH ₂) ₃ -N/(CH ₂) ₃ NH-BOC ₇ ₂		AC ₁₋₅			-NH-(CH ₂) ₃ -N/(CH ₂) ₃ NH ₂ -7	
48 TGAC ₂₋₅	NH ₂ (CH ₂) ₃ -N/(CH ₂) ₃ NH-BOC ₇ ₂		AC ₂₋₅			-NH-(CH ₂) ₃ -N/(CH ₂) ₃ NH ₂ -7	
49 TGAC ₂	NH ₂ (CH ₂) ₃ -N/(CH ₂) ₃ NH-BOC ₇ ₂		AC ₂			-NH-(CH ₂) ₃ -N/(CH ₂) ₃ NH ₂ -7	
50 DTG	NH ₂ (CH ₂) ₃ -N/(CH ₂) ₃ NH-BOC ₇ ₂		H	H	H	-NH-(CH ₂) ₃ -N/(CH ₂) ₃ NH ₂ -7	
51 DTG	NH ₂ (CH ₂) ₃ -N/(CH ₂) ₃ NH ₂ -7-(CH ₂) ₃ N(CH ₃) ₂		H	H	H	-NH-(CH ₂) ₃ -N/(CH ₂) ₃ NH ₂ -7-(CH ₂) ₃ N(CH ₃) ₂	

75

TABLE VI: (continued)

Comp. No.	Teicoplanin starting material	Reagents	End product of formula I (R = H)				
			Amine	A/AC	B	M	Y
52	DIG	NH ₂ (CH ₂) ₃ -N(CH ₂) ₃ NH ₂ -I-(CH ₂) ₂ N(C ₂ H ₅) ₂	H	H	H	H	-NH-(CH ₂) ₃ -N(CH ₂) ₃ NH ₂ -I-(CH ₂) ₂ N(C ₂ H ₅) ₂
53	DIG	NH ₂ (CH ₂) ₃ -N(CH ₂) ₃ -N(CH ₃) ₂	H	H	H	H	
54	DIG	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₉ -NH(CH ₂) ₃ NH ₂	H	H	H	H	-NH-(CH ₂) ₃ -NH(CH ₂) ₉ -NH(CH ₂) ₃ NH ₂
55	DIG	NH ₂ (CH ₂) ₃ -NH(CH ₂) ₁₀ -NH(CH ₂) ₃ NH ₂	H	H	H	H	-NH-(CH ₂) ₃ -NH(CH ₂) ₁₀ -NH(CH ₂) ₃ NH ₂

HPLC Analysis is carried out with a Varian mod. 5000 LC pump equipped with a 20 microliter loop injector Rheodyne mod. 7125 and a UV detector at 254 nm.

5 Columns: pre-column (1.9 cm.) Hibar LiChro Cart 5-4 (Merck) pre-packed with Lichrosorb RP-8 (20-30 micrometer) followed by a column Hibar RT 250-4 (Merck) pre-packed with LiChrosorb RP-8 (10 micrometer).

Eluents: A, 0.2% aq. HCOONH₄; B, CH₃CN.

10 Flow rate: 2 mL/min.

Injection: 20 microliter.

Elution: linear gradient from 20 to 60% of B in A in 30 min. The retention times of some representative compounds are reported in TABLE VII.

15 Acid-Base Titrations. The products are dissolved in MCS (methylcellosolve):H₂O 4:1 (v/v), then an excess of 0.01 M HCl in the same solvent mixture is added and the resulting solutions are titrated with 0.01N NaOH. Equivalent weight of some representative compounds are reported in TABLE VIII.

20 ¹H-NMR spectra at 500 MHZ are recorded in the temperature range from 20°C to 30°C on a Bruker AM 500 spectrometra in DMSO-D₆ with tetramethylsilane (TMS) as the internal reference (delta = 0.00 ppm). Table IX 25 reports the most significant chemical shift (delta ppm) of some representative compounds.

Table VII

Retention times (t_R) determined as described above
for some representative compounds of the invention

5

	Compound No.	t_R (min)
10	2	15.6
	3	16.1
	4	16.1
	6	15.7
15	7	16.9
	8	16.8
	9	16.9
	10	17.1
	11	17.1
20	14	15.7
	15	17.0
	21	12.4
	22	12.7
	23	12.8

25

Table VII (continued)

5

	Compound No.	t_R (min)
	24	15.8
	25	15.7
10	26	15.7
	27	15.9
	29	12.5
	30	15.9
	31	13.6
15	33	15.4
	34	14.4
	35	13.8
	36	13.4
	39	13.1
20	41	11.0
	44	14.2
	46	6.7
	47	14.4
	50	13.6
25	51	13.9
	52	14.2
	53	13.1
	54	19.5
	55	21.0

30

Table VIII

Yields and equivalent weight (EW) of some representative compounds of formula I. Between brackets
 5 are indicated the number of equivalents titrated for each molecule.

	Compound No.	Yield %	EW
10			
15	2	72	675 (x3)
	3	61	690 (x3)
	4	59	681 (x3)
	6	67	681 (x3)
	7	81	506 (x4)
	8	78	511 (x4)
20	9	81	512 (x4)
	10	75	510 (x4)
	11	70	504 (x4)
	14	86	492 (x4)
	15	81	496 (x4)
	21	81	441 (x3)
25	22	63	442 (x3)

Table VIII (continued)

5

	Compound No.	Yield %	EW
10			
15	23	86	456 (x3)
	24	84	348 (x4)
	25	83	349 (x4)
	26	86	361 (x4)
	27	81	351' (x4)
	29	88	369 (x4)
20	30	83	349 (x4)
	39	96	421 (x4)
	41	91	408 (x4)
	44	83	386 (x4)
	46	28	1910 (x2)
	47	61	499 (x4)
25	48	60	486 (x4)
	49	66	487 (x4)
	50	46	356 (x4)
	51	34	363 (x4)
	52	39	350 (x4)
	53	56	475 (x3)
30	54	55	360 (x4)
	55	51	355 (x4)

Table IX

Significant ^1H -NMR assignments of some representative compounds recorded in DMSO-d_6 with 5 tetramethylsilane as internal reference (delta = 0.00 ppm).

10 Compound 2 3.61, 2.95 (CH_2 -side chain); 0.83, 1.18, 1.46, 2.02 (acyl chain); 4.18-5.62 (peptidic CH's); 1.89 (acetylglucosamine); 6.18-8.45 (aromatic protons and peptidic NH's)

15

Compound 4 3.45, 2.82, 2.63, 2.08, 1.66 (CH_2 -side chain); 0.87, 1.23, 1.45, 2.01 (acyl chain); 1.88 (acetylglucosamine); 20 4.15-5.71 (peptide CH's); 6.26-8.56 (aromatic protons and peptidic NH's)

25

Compound 6 3.52, 2.73, 2.58, 1.91, 1.56 (CH_2 -side chain); 0.84, 1.15, 1.46, 2.02 (acyl chain); 1.82 (acetylglucosamine); 3.42 (mannose); 4.15-5.69 (peptidic CH's); 6.29-8.53 (aromatic protons and peptidic NH's)

30

Table IX (continued)

Compound 7	3.68, 3.12, 2.98, 2.05 (CH_2 -side chain); 0.84, 1.16, 1.44, 2.01 (acyl chain); 1.89 (acetylglucosamine); 3.42 (mannose); 4.13-5.58 (peptidic CH's); 6.21-8.53 (aromatic protons and peptidic NH's)
5	
10 Compound 8	3.68, 2.92, 1.98 (CH_2 -side chain); 0.82, 1.25, 1.43, 2.01 (acyl chain); 1.87 (acetylglucosamine); 4.17-5.65 (peptidic CH's); 6.26-8.57 (aromatic protons and peptidic NH's)
15	
Compound 9	3.48, 2.98, 1.98 (CH_2 -side chain); 0.82, 1.12, 1.43, 2.01 (acyl chain); 1.86 (acetylglucosamine); 4.16-5.62 (peptidic CH's); 6.18-8.58 (aromatic protons and peptidic NH's)
20	
Compound 11	3.71, 2.93, 1.98, 1.73 (CH_2 -side chain); 0.83, 1.23, 1.47, 2.02 (acyl chain); 1.89 (acetylglucosamine); 4.13-5.58 (peptidic CH's); 6.21-8.62 (aromatic protons and peptidic NH's)
25	

Table IX (continued)

5	Compound 15	3.52, 3.13 (CH_2 piperazine); 3.42-2.07 (CH_2 -side chain); 0.83, 1.16, 1.45, 2.02 (acyl chain); 1.88 (acetylglucosamine); 4.16-5.32 (peptidic CH's); 6.18-8.53 (aromatic protons and peptidic NH's)
10	Compound 21	3.52, 3.04, 2.81 (CH_2 -N); 4.15-5.62 (peptidic CH's); 6.18-8.62 (aromatic protons, peptidic NH's)
15	Compound 22	3.17, 2.93, 2.83, 1.87, 1.80 (CH_2 -side chain); 4.15-5.61 (peptidic CH's); 6.18-8.53 (aromatic protons and peptidic NH's)
20	Compound 23	3.42, 2.98-2.71, 1.72, 1.61 (CH_2 spermidine); 4.15-5.63 (peptidic CH's), 6.19-8.43 (aromatic protons and peptidic NH's)
25	Compound 24	3.38, 3.12, 2.98 (CH_2 -N), 1.89 (CH_2); 4.17-5.58 (peptidic CH's); 6.18-8.48 (aromatic protons, peptidic NH's)
30		

Table IX (continued)

- Compound 25 3.34, 3.08, 2.84, 1.78 (CH_2 -side chain);
5 4.16-5.58 (peptidic CH's); 8.31-8.48
 (aromatic protons and peptidic NH's)
- Compound 26 3.36, 2.98, 2.87 ($\text{CH}_2\text{-N}$); 1.89, 1.77
10 (CH_2); 4.15-5.62 (peptidic CH's);
 6.18-8.42 (aromatic protons, peptidic
 NH's)
- Compound 27 3.12, 3.03-2.82, 1.88, 1.81, 1.65
15 (CH_2 -side chain); 4.15-5.62 (peptidic
 CH's); 6.19-8.41 (aromatic protons and
 peptidic NH's)
- 20 Compound 29 3.48, 3.12, 2.83, 2.64 ($\text{CH}_2\text{-N}$); 4.18-5.61
 (peptidic CH's); 6.21-8.70 (aromatic
 protons and peptidic NH's)
- 25 Compound 30 3.49, 3.11, 2.95, 2.85, 1.85 (CH_2 side
 chain); 4.18-5.81 (peptidic CH's);
 6.21-8.56 (aromatic protons, peptidic
 NH's)

Table IX (continued)

5	Compound 31	3.01 N(CH ₃) ₃ [⊕] ; 2.95, 2.21, 1.68 (CH ₂ -side chain); 2.28, 2.06 (NCH ₃); 0.83, 1.21, 1.46 (acyl chain); 1.86 (acetylglucosamine); 3.48 (mannose); 4.17-5.82 (peptidic CH's); 6.14-8.62 (aromatic protons and peptidic NH's)
10	Compound 34	3.03-2.81; 1.82, 1.62, 1.43 (CH ₂ -side chain); 2.01 (CH ₃ -CH), 1.23 (CH ₃); 4.13-5.62 (peptidic CH's); 6.19-8.47 (aromatic protons and peptidic NH's)
15	Compound 35	5.25 (CH ₂ -O), 4.51 (CH ₂ OH); 2.06 (NCH ₃); 0.81, 1.23, 1.45, 2.02 (acyl chain); 1.87 (acetylglucosamine); 4.12-5.69 (peptidic CH's); 6.14-8.48 (aromatic protons and peptidic NH's)
20	Compound 36	5.22 (CH ₂ -O); 3.72, 2.82 (CH ₂ -side chain); 1.93 (NCH ₃); 0.83, 1.14, 1.43, 2.01 (acyl chain); 1.87 (acetylglucosamine); 4.18-5.72 (peptidic CH's); 6.31-8.45 (aromatic protons and peptidic NH's)
25	Compound 44	3.68, 3.34, 2.95, 1.99, 1.84 (spermine); 1.87 (acetylglycosamine); 4.18-5.61 (peptidic CH's); 6.19-8.56 (aromatic protons and peptidic NH's)
30		
35		

Table IX (continued)

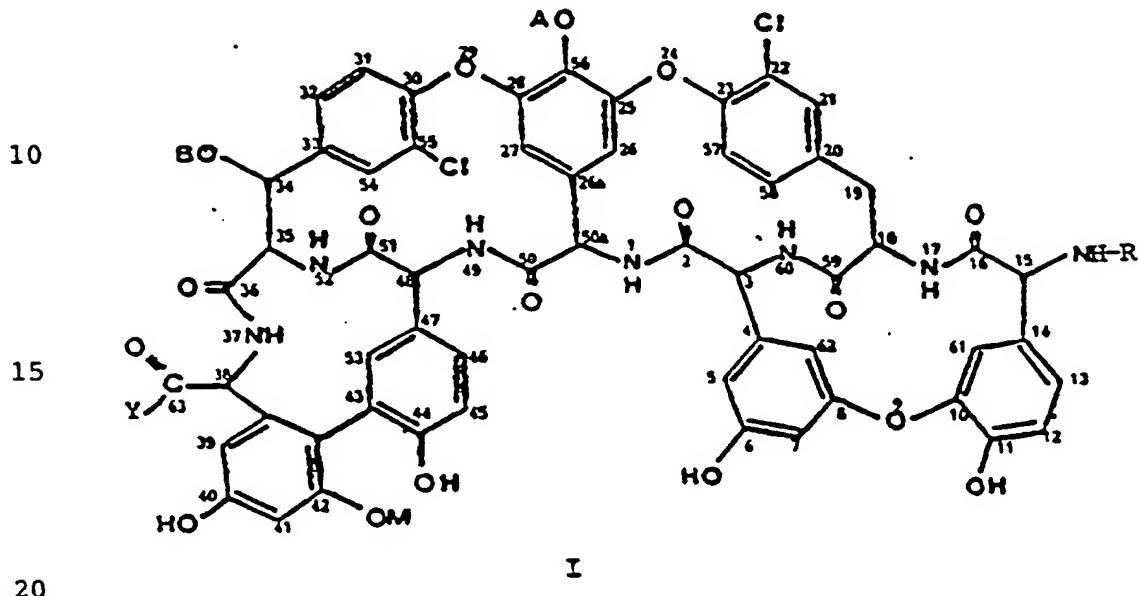
5	Compound 39	3.71, 3.37, 2.98, 1.98, 1.82 (spermine); 1.86 (acetylglucosamine); 3.44 (mannose); 4.16-5.58 (peptidic CH's); 6.29-8.54 (aromatic protons and peptidic NH's).
10	Compound 46	3.41, 3.23, 3.16, 2.94 (CH_2 -side chain); 1.12, 1.23 (CH_3 -side chain); 0.81, 1.15, 1.46, 2.00 (acyl chain), 1.86 (acetylglucosamine); 4.16-5.59 (peptidic CH's); 6.23-8.01 (aromatic protons, peptidic NH's).
15	Compound 47	3.45, 3.09, 2.35, 1.84 (CH_2 -side chain); 0.82, 1.14, 1.46, 2.03 (acyl chain); 1.86 (acetylglucosamine); 4.12-5.62 (peptidic CH's); 6.23-8.43 (aromatic protons, peptidic NH's).
20	Compound 50	3.43, 3.24, 2.85, 1.84 (CH_2 -side chain); 4.12-5.62 (peptidic CH's); 6.21-8.51 (aromatic protons, peptidic NH's).
25	Compound 51	3.45, 3.39, 3.05, 2.91, 2.12, 1.98 (CH_2 -side chain); 2.74 (NCH_3); 4.13-5.59 (peptidic CH's); 6.18-8.61 (aromatic protons, peptidic NH's).
30		

Table IX (continued)

5	Compound 52	3.48, 3.12, 1.89 (CH_2 -side chain); 1.18 (2CH_3 -ethyl); 4.12-5.61 (peptidic CH's); 6.20-8.52 (aromatic protons, peptidic NH's).
10	Compound 53	3.42, 3.39, 3.30, 1.98 (CH_2 -pip., CH_2 -propyl); 2.75 (NCH_3); 4.05-5.63 (peptidic CH's); 6.32-8.52 (aromatic protons, peptidic NH's).
15	Compound 55	3.32, 2.98, 2.76, 1.86, 1.52, 1.24 (CH_2 -side chain); 4.13-5.62 (peptidic CH's); 6.31-8.42 (aromatic protons, peptidic NH's).
20	Compound 54	3.37, 3.34, 3.07, 2.86, 2.78, 1.75, 1.59, 1.26 (CH_2 -side chain); 4.14-5.61 (peptidic CH's); 6.21-8.34 (aromatic protons, peptidic NH's).
25		

Claims

5 1. A teicoplanin amide derivative of formula I



20

wherein

25 R represents hydrogen or a protecting group of
the amine function;

Y represents a compound of formula

-NR₁-alk₁-X-alk₂-T_p-T-alk₃-T_q-W

wherein

R₁ represents hydrogen or (C₁-C₄) alkyl;

30 alk₁, alk₂ and alk₃ each independently represents a
linear or branched alkylene of 2 to 10 carbon atoms;

T_p represents an integer comprised between 1 and
50;

T_q represents an integer comprised between 0 and

35 12;

X represents a $-NR_2-$ group or an oxygen atom wherein R_2 represents hydrogen, (C_1-C_4) alkyl, a group $alk_4NR_3R_4$ wherein alk_4 represents a linear or branched alkylene of 2 to 4 atoms, R_3 is hydrogen or (C_1-C_4) alkyl and R_4 is hydrogen, (C_1-C_4) alkyl or a 5-6 membered cycloalkyl; or R_1 and R_2 taken together represent a (C_2-C_4) alkylene moiety connecting the two nitrogen atoms with the proviso that in such case p is 1;

T represents a $-NR_5-$ group or an oxygen atom wherein R_5 is hydrogen, (C_1-C_4) alkyl a group $alk_5NR_6R_7$ wherein alk_5 represents a linear or branched alkylene of 2 to 4 atoms, R_6 is hydrogen or (C_1-C_4) alkyl and R_7 is hydrogen, (C_1-C_4) alkyl or a 5-6 membered cycloalkyl; or R_2 and R_5 taken together represent a (C_2-C_4) alkylene moiety connecting the two nitrogen atoms with the proviso that in such case both p and q are 1;

W represents hydroxy, NR_8R_9 wherein R_8 is H or (C_1-C_6) alkyl and R_9 is H, (C_1-C_6) alkyl, a 5-6 membered cycloalkyl, $COOR_{10}$ wherein R_{10} represents (C_1-C_6) acyloxy- (C_1-C_4) alkyl, and the group $\overset{\oplus}{N}R_{11}R_{12}R_{13}\overset{\ominus}{A}M$ wherein R_{11} , R_{12} and R_{13} each independently represents (C_1-C_4) alkyl and A^-M is an anion derived from a pharmaceutically acceptable acid; with the proviso that when simultaneously X is NR_2 , p is 1 and q is zero, then W is different from hydroxy;

A represents H or $-N/(C_9-C_{12})$ aliphatic acyl- β -D-2-deoxy-2-aminoglucopyranosyl,

B represents hydrogen or N-acetyl- β -D-2-deoxy-2-aminoglucopyranosyl;

M represents hydrogen or alpha-D-mannopyranosyl

and the pharmaceutically addition salts thereof;

with the further proviso that B represents hydrogen only when A and M are simultaneously hydrogen.

5

2. A compound of claim 1 wherein the (C_9-C_{12}) aliphatic acyl radicals of the symbol A is one of the following:

10 (Z)-4-decenoyl, 8-methylnonanoyl, decanoyl, 8-methyldecanoyl, 9-methyldecanoyl, 6-methyloctanoyl, nonanoyl, 10-methylundecanoyl and dodecanoyl.

15 3. A compound of claim 1 wherein X and/or T represent $-NR_2-$ and/or $-NR_5-$; alk₁, alk₂, alk₃, represent a (C_2-C_{10}) linear chain, p is an integer comprised between 1 and 5, and q is an integer comprised between 0 and 12.

20

4. A compound of claim 1 wherein both X and T represent oxygen atoms and p and q are such that p + q is comprised between 2 and 50.

25

5. A compound of claim 1 wherein X represents a $-NR_2-$ group wherein R₂ is hydrogen, a (C_1-C_4) alkyl or a alk₄NR₃R₄ being alk₄, R₃ and R₄ defined as in claim 1.

30

6. A compound of claim 1 wherein p is 1 and X is $-NR_2-$ wherein R₂ taken together with R₁ represent a (C_2-C_3) alkylene moiety connecting the nitrogen atoms.

35

7. A compound of claim 1 wherein p is 1, q is 1 and X and T are $-NR_2-$ and $-NR_5-$ respectively, wherein R_2 and R_5 taken together represent a (C_2-C_3) alkylene moiety connecting the nitrogen atoms.

5

8. A compound of claim 6 or 7 wherein the alkylene moiety is a group $-CH_2-CH_2-$.

10

9. A compound of claim 1 wherein X and T are oxygen atoms and W is hydroxy or $-NR_8R_9-$ wherein R_8 is hydrogen or (C_1-C_4) alkyl and R_9 is hydrogen, (C_1-C_4) alkyl, cyclopentyl or cyclohexyl.

15

10. A compound of claim 1 wherein W represents $-NR_8R_9-$ wherein R_8 is as defined and R_9 is $COOR_{10}$ being R_{10} a (C_1-C_6) acyloxy- (C_1-C_4) alkyl group.

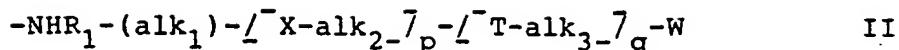
20

11. A compound of claim 10 wherein the (C_1-C_4) alkyl group of the (C_1-C_6) acyloxy- (C_1-C_4) alkyl moiety is a methylene optionally substituted with a (C_1-C_3) linear or branched alkyl chain.

30

12. A process for preparing a teicoplanin derivative of claim 1 which comprises amidation of the corresponding carboxylic teicoplanin starting material of the formula I wherein A, B, M have the same meaning

as in claim 1 and Y is OH with an amine of the formula



5

wherein R₁, alk₁, alk₂, alk₃, X, T, p, q, and W are defined as in claim 1.

10 13. A process as in claim 12 wherein the amidation process is carried out in an inert organic solvent in the presence of a condensing agent selected from (C₁-C₄)alkyl, phenyl or heterocyclic phosphorazidates at a temperature comprised between 0°C and 20°C.

15

14. A process as in claim 12 wherein the amidation process is carried out by converting the carboxylic starting material in its corresponding activated ester 20 preferably protected on the N¹⁵-amino function and the activated ester is reacted with a molar excess of an amine of formula II of claim 12 in the presence of an organic polar solvent at a temperature between 5°C and 60°C, preferably between 10 and 30°C.

25

15. A process as in claim 14 where the activated ester is the cyanomethyl ester and its molar proportion against the amine ranges from 1:5 to 1:30.

30

16. A process as in claim 15 where the cyanomethyl ester is prepared by reacting the carboxylic acid starting material preferably protected on the N¹⁵-amino 35 function with an about 20 to 30-time molar excess of

chloroacetonitrile in the presence of an inert organic solvent and a base which does not interfere with the reaction course at a temperature between 5°C and 60°C, preferably between 10°C and 30°C.

5

17. A process for preparing a compound of formula I wherein W is $-NR_8R_9-$ wherein R₈ is defined as in claim 1 or in claim 2, R₉ is COOR₁₀ and R₁₀ is a
10 (C₁-C₆)acyloxy-(C₁-C₄)alkyl which consists in reacting a N¹⁵ protected derivative of the N⁶³-amide wherein W is -NHR₈- wherein R₈ is defined as in claim 1 with an alpha-acyloxy-alkyl para-nitrophenyl carbonate in the presence of an anhydrous alkaline carbonate.

15

18. A process as in claims 12 to 17 which further comprises the additional step of removing the N¹⁵ protecting group.

20

19. A process for preparing a teicoplanin amide derivative of claim 1 which consists in reacting a N¹⁵ protected derivative of a N⁶³ amide of formula I
25 wherein Y is -NR₁alk₁XH or NR₁-alk₁-X-alk₂-⁷p-TH with a reactant of the formula r-alk₂-⁷p-T-alk₃-⁷q-W or r-alk₃-⁷q-W, respectively, wherein the symbol R₁, alk₁, alk₂, alk₃, X and T are the same as in claim 1, r represents halo, methanesulfonyl or tosyl, in the presence of an acid acceptor in an inert solvent.

20. A process for the manufacture of the compounds of the formula I wherein A is hydrogen, B is
35 N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl and M is

alpha-D-mannopyranosyl which consists in hydrolyzing the corresponding amide compounds of formula I wherein A is N- $\text{C}_9\text{-}\text{C}_{12}$) aliphatic acyl- β -beta-D-2-deoxy-2-amino-glucopyranosyl, B is N-acetyl-beta-D-2-deoxy-2-amino-glucopyranosyl and M is alpha-D-mannopyranosyl with concentrated aqueous organic acid at about room temperature, preferably with aqueous trifluoroacetic acid at a concentration between 75% and 95% at a temperature comprised between 10°C and 50°C.

10

21. A process for preparing the compounds of formula I wherein both A and M are hydrogen and B is N-acetyl-beta-D-2-deoxy-2-aminoglucopyranosyl which consists in contacting the corresponding amide compounds of formula I wherein A is N- $\text{C}_9\text{-}\text{C}_{12}$) aliphatic acyl- β -beta-D-2-deoxy-2-aminoglucopyranosyl, B is N-acetyl- β -beta-D-2-deoxy-2-aminoglucopyranosyl and M is alpha-D-mannopyranosyl with a strong acid in the presence of a polar aprotic organic solvent selected from ethers, ketones and mixture thereof which are liquid at room temperature.

25 22. A substance as in claim 1 for use as a medicament.

30 23. Use of a substance of claim 1 for the manufacture of a medicament for combatting bacterial infections.

35 24. A pharmaceutical formulation containing a compound of claim 1 as the active ingredient.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/00400

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: C 07 K 9/00, A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched †

Classification System ‡	Classification Symbols
IPC⁵	C 07 K, A 61 K
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched §	

III. DOCUMENTS CONSIDERED TO BE RELEVANT*

Category ¶	Citation of Document, with indication, where appropriate, of the relevant passages ‡‡	Relevant to Claim No. †‡
X	WO, A, 88/06600 (GRUPPO LEPETIT S.p.A.) 7 September 1988 see the whole document --	1-3,5
X	EP, A, 0218099 (GRUPPO LEPETIT S.p.A.) 15 April 1987 see the whole document --	1-3,5
X	J. Med. Chem., vol. 32, February 1989, American Chemical Society, A. Trani et al.: "Deaminoteicoplanin and its derivatives. Synthesis, antibacterial activity, and binding strength to Ac-D-Ala-D-Ala", pages 310-314, see page 311, figure 1	1

* Special categories of cited documents: ¶

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

25th May 1990

Date of Mailing of this International Search Report

03.07.90

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorised Officer

MISST. TAZELAAR

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

EP 9000400
SA 34910

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 26/06/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 8806600	07-09-88	AU-A-	1363288	26-09-88
		EP-A-	0340245	08-11-89
EP-A- 0218099	15-04-87	AU-A-	6259286	19-03-87
		JP-A-	62061998	18-03-87